PATHOPHYSIOLOGICAL EFFECT OF AQUEOUS EXTRACT OF *MORINGA OLEIFERA* LEAVES ON *CLARIAS GARIEPINUS* CHALLENGED WITH *ICHTHYOPHTHIRIUS MULTIFILLIS*

BY

IKELE, CHIKA BRIGHT

REG NO: PG/Ph.D/11/58649

A PROJECT SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN ZOOLOGY (FISHERIES BIOLOGY FACULTY OF BIOLOGICAL SCIENCES, UNIVERSITY OF NIGERIA NSUKKA

SUPERVISOR: PROF. B. O. MGBENKA

JANUARY, 2017

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TITLE PAGE

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CERTIFICATION

Ikele, Chika Bright, a post graduate student in the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka with Registration Number PG/Ph.D/11/58649 has satisfactorily completed the requirements for research work for the degree of Doctor of Philosophy (Ph.D) in Zoology (Fisheries Biology). The work embodied in this thesis is original and has not to our knowledge been submitted in part or full for any other diploma or degree in or any other University.

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PROF. B. O. Mgbenka Date

Supervisor

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Prof. B. O. Mgbenka Date

Head of Department

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External examiner: Date.

DEDICATION

This work is dedicated to the Almighty God for His infinite love for me

ACKNOWLEDGMENTS

I am most grateful to my supervisor, Prof. B. O. Mgbenka for his wonderful effort, and excellent supervision made to see this work is done well. Words only are grossly insufficient in expressing my immense and profound gratitude to him. He was indeed the latent force behind the prompt success of this research work, through his ever ready assistance, prodigious encouragement and constructive advice that sustained me throughout the duration of this project.

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ABSTRACT

Icthyophthirius multifilis (Ich), a dangerous ectoparasite causes white spot disease and economic losses in fish farms. A majority of the chemotherapeutic agents of ichthyophthiriasis are either pollutants of the environment, banned or lack efficacy. The present work was focused on evaluating different immersion treatments of ichthyophthiriasis of African catfish (*Clarias gariepinus*) with non-chemical and environmental friendly parasiticide, *Moringa oleifera.* A total of 800 apparently healthy parasites-free post juvenile catfish were disinfected with 0.5% potassium permanganate. They were challenged with about 44,000 theronts obtained through serial passages by cohabitation. Seven groups (A - G) of post juvenile fish were used in the experiment in randomized complete block design with groups A, B and C as the uninfected control, Ich-infested not treated and Ich-infested treated with standard drug (fish cure), respectively, whereas, the groups D - G exposed to aqueous extract of *M. oleifera* leaves (EMOL) in dip (1,500 mg/L - 4,500 mg/L for 1h), short term (150 mg/L ï 450mg/L for 24h ï 96h) and prolonged bath treatment (15 mg/L ï 45 mg/L for 5 ï 15days). The adult parasite (trophont) burdens on the experimental fish were determined in one time point, two time points and four time points. Gross examination, histopathological alterations, hematological parameters, antioxidants enzymes and biochemical parameters were determined using standard protocols. Data obtained were subjected to analysis of variance at 5% probability while differences between means were partitioned using the Duncan New Multiple Rang Test. The results showed almost complete elimination of the trophonts both in the gills and the body smear of *Ichthyophthirius multifilis* infested fish. The observed curative potential of the various extract-treated groups and standard drug-treated groups were all significantly different from the infected non-treated group ($p < 0.05$). The knockout efficacy in short term bath treatment was significantly different ($p<0.05$) from the control. The ich-infested fish had visible lesions and histop athological alterations in the gills and skin among the groups. Packed cell volume, haemoglobin, white blood cell, red blood cell, differential count and the blood bicarbonate, chloride, potassium and sodium had significant ($p < 0.05$) changes among the experimental groups. Some of the observed changes during the immersion treatments did not differ significantly with the fish cure treatment ($p > 0.05$).The antioxidant parameters (catalase, superoxide dismutase, glutathione peroxidase and lipid peroxidations in the gills and muscles of ich-infested fish among the groups) and biochemical parameters (total protein, albumin, globulin, urea, creatinine, lactate dehydrogenase, aspartate and alanine aminotransferase) showed significant changes ($p < 0.05$) among the ich infested fish. In conclusion, aqueous extract of *Moringa oleifera leves* has proved to be an effective botanical in controlling the skin-parasitic ciliate, *Ichthyophthirius multifilis* of *Clarias gariepinus*.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Ichthyophthirius multifilis a protozoan parasite that is responsible for white spot (Ichthyophthiriasis) is a prevalent ectoparasite mostly affecting cultured and aquarium fishes. The morbidity rate due to this disease may reach up to 100%, causing great economic losses in fish farms. Ichthyophthiriasis has also been known as sand grain, gravel or Ich disease (Richard and Medunald, 1998; Traxler *et al.,* 1998; Xu *et al.*, 2002). This dangerous ectoparasite mainly attacks skin, fins, gills and buccal cavity and is characterized by the presence of white spots all over the external body surface.

The parasite is commonly distributed, occurring in tropical, subtropical and temperate regions and extending north to the Arctic Circle (Mathew *et al*, 1994). It causes severe epizootics among different fish species in aquaria, hatcheries, and ponds, as well as in wild fish population (Ezz El-Dien *et al.,* 1998; Kim *et al.,* 2002; Thilakaratne *et al.,* 2003). Naturally occurring outbreaks of ichthyophthiriasis in wild fish populations can yield devastating effects for example, natural outbreaks of the Ich was blamed for the deaths of 18 million *Orestias agassi* in Lake Titicaca, Peru (Wurtsbaugh and Tapia, 1997). In intensive aquaculture systems, Ich epizootics are more common (Valtonen and Keranen, 1997) due to the confinement of fish under stressful condition and the exponential increase in parasite numbers (Clark *et al.,* 1998). Epizootics were reported in China as early as the tenth century (Hines and Spira, 1974a). Disease and aquaculture species caused by parasites and infectious pathogens have attracted the attention of veterinarian and fish biologists from the early days of aquaculture investigations. A number of prophylactic and curative measures have also been suggested, although many of the chemicals have not been cleared for use in some countries. With increasing investment in aquaculture and closer examination of factors that contribute to the risks faced by an aquaculturist, the concept of integrated health protection measures have developed in recent years. Similarly, experience of fish farming in the tropics has brought into focus the public health aspects of fish farm development and the possible role of aquatic farming in the spreading of communicable diseases. This fish health and disease control are now viewed from different angles that include environmental protection and pollution and pollution control, human health and epidemiology, site selection and culture technologies, monitoring, and sanitation of aquaculture facilities, diagnosis and treatment of diseases of cultured species, avoidance of nutritional diseases, prevention of epidemics of mortality in cultured facilities, formulation and implementation of

regulatory measures to control national and international spread of communicable diseases, development of disease-resistant strains through genetic selection and hybridization and individual and mass immunization of cultured species (Xu *et al.*, 2002).

From the foregoing, infection by ciliate protozoan *Ichthyophthirius multifiliis* Fouquet, 1876 causes significant economic losses in fresh water aquaculture worldwide. Following the ban of the use of malachite green for treating food fish, there has been extensive research aimed at identifying suitable replacements. Current treatments used are formaldehyde, sodium chloride, copper sulphate, potassium permanganate. Other environmentally friendly drugs includes; humic acid, potassium ferrate (VI), broponol, peracetic acid and toltrazuril. However, further investigation is required to optimize the treatment and to establish precise protocol in order to minimize the quantity of drugs employed whilst ensuring the most efficiency and the most efficacious performance. Moreover, some of the chemicals are banned for aquaculture usage due to their adverse environmental and health effects (cancer).

However, there is limited knowledge about anti-parasiticidal activity of herbs as a natural treatment for fish parasites. The use of herbs in aquaculture industry was improved in recent ten years, because of chemical pollutions caused by the use of commercial medicines. To the best of my knowledge first time *Moringa oleifera* has not being used as a botanical treatment in controlling Ich parasites in fish. *Moringa oleifera* is a highly valued plant which has an impressive range of medicinal uses with high nutritional value. It provides a rich and rare combination of zeatin, quercetin and beta-sitosterol. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods can be effective in treatment of *Pseudomonas aeruginosa* bacterium which can cause diseases in both animal and humans (Spillotis, 1998). Similarly, study on the ethanolic and ethyl acetate extracts of *Moringa oleifera* showed antipyretic/wound healing activity in rats (Udupa, 1994).

Justification of the study

Majority of chemotherapeutants lack efficacy for treating *Ichthyophthirius multifilis* or they are not safe for human health and environment. Therefore, there is urgent need to discover alternative parasiticides that are more environmentally friendly as alternatives for the control of *I. multifiliis* infection. This work is focused on evaluating the different bath treatments (dip, short term and prolonged term) of *Clarias gariepinus* infected with *Ichthyophthirus multifiliis* and treated with aqueous extracts of *Moringa oleifera* leaves.

1.1.2. Aims and Objectives of the Study

The aims of the study was to Evaluate the effect of aqueous extracts *Moringa oleifera* leaves on the bath treatments (Dip, short-term and prolonged bath) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis*,

1.1.2.1. Specific objectives of the study

The specific objectives were to:

i. assess the anti-parasiticidal potential of aqueous extracts of *M. oleifera* leaves on the parasite load of *C. gariepinus* infested with Ich.
ii. determine the anti-protozooal activity of aqueous extracts of *M. oliefera* on the infective stage (theronts).
iii. determine the morphological alterations of treated theronts.
iv. evaluate the infestation potential/or viability of infective theronts pretreated with *M. oleifera* for 14 days.
v. determine the infective prevalence and intensity of theronts pre-treated for 1h with aqueous extracts *M. oleifera* leaves.
vi. examine the gross morphology (skin, gills and fins) and histopathological alterations in the gills and skin of the infested fish, *C. gariepinus*, treated in dip (1 h, short term (24 - 96 h) and prolonged baths (5 - 15 days) with aqaeous extracts of *M. oleifera* leaves.
vii. assess the proximate compositions of Ich infested fish treated in dip (1hr), short-term (24 - 96 h) and prolonged bath (5 - 15 days) with aqueous extracts of *M. oleifera* leaves.
viii. assess the effect of *Moringa oleifera* aqaeous extracts as non chemical drug in the haematological parameters and blood electrolyte of *Clarias gariepinus* exposed to bath (dip, short term and prolonged bath) treatments.
ix. evaluate the antioxidant defense enzymes; catalase, superoxide dismutase, gluthathione peroxidase, lipid peroxidation (malanoaldehyde) as a marker for cellular damage of *C. gariepinus* infested with Ich and treated with aqueous extracts of *M. oleifera* in the dip (1 h), short (24-96 h) and prolonged bath treatments (5 - 15 days).
x. determine myeloperoxidase enzyme activity of *C. gariepinus* infested with Ich and treated with aqueous extracts of *M. oleifera* leaves in the dip (1 h), short (24 - 96 h) and prolonged bath treatments (5 - 15 days).

xi. determine the serum biochemical parameters of *C. gariepinus* infected with *I. multifilis* and treated with aqueous extracts of *Moringa oleifera* leaves in dip (1 h), short term (24 -96 h) and prolonged bath treatments (0-15 days).

1.2. Literature Review

A number of studies have indicated that sublethal infections in the host are able to induce an acquired resistance against re-infection. The mechanisms of the protective response for host resistance have not been fully elucidated, however (Woo, 1995) suggested that immunoprophylactic measures should be considered in order to control Ich infection and one of these possibilities could be vaccination with Ich preparation. Antibodies against antigens of Ich were found in both serum and mucous collected form Ich immune fish. Some reports showed that serum antibody levels in fish did not correlate with induced protections after immunization (Clark *et al.,* 1988). These suggest that there may be other protective mechanisms against these pathogens in addition to the systemic immune response. Trials were carried out per collection of trophonts from infected fish and subsequent production of tomonts and theronts for vaccine. Applying this method resulted in a clear protection against re-infection and also activated host factors following treatment of infection with skin parasitic mongeneas. Also this method was reported to reduce the establishment of Ich in rainbow trout (Woo, 1995).

1.2.1. Geographical distribution and the host range of parasites.

1.2.1.1. Geographical distribution

Ichthyophthirius multifiliis is a cosmopolitan parasite of fishes. The infection has been reported from virtually all areas where fishes are cultured including the tropics and subarctic regions. The organism was first described in detail by Fouquet in 1876 in France (Woo, 1995). Parasitic incidence of Ich has been reported by (Adeyemo and Falaye, 2007) in the Ministry of Agriculture and National Resources, Agodi, Ibadan where they opined about 3.7% of that *C. gariepinus* outbreak. Similarly, Adedeji (2012) reported prevalence of the following diseases; tapeworm infestation, fish lice, saprolegnia, Ichthyophiriasis, furunculosis among others in the South Western Nigeria, Oyo State, Ibadan.

1.2.1.2 Host Range

I. multifiliis appears to parasitize all fresh water fishes. There are no records of species with natural resistance (Ventura and Paperna, 1985).

However, there are suggestions of variation in the degree of susceptibility between fishes. The variations may depend on factors such as genetic background, physiological status of the fishes, parasite stages and environmental conditions.

Epizootics often occur in only one species of fish in a population. Elser (1999) reported on an outbreak in a reservoir in Maryland. The disease affected predominantly Yellow perch (*Perca flavesceis*). Allison and Kelly (2004) reported epizootics in rivers of North Western Alabama. The majority of infected fishes were gizzard shad (*Dorosoma cepedianum*) and threadfin shad (*D. petenense*). In an outbreak reported in Ketuncky, the only fishes infected were blackstripe top minnows (*Fundulus notatus*).

The occurrence of epizootics in only one or a few species in a mixed fish population may not indicate genetic variation in resistance, but rather different physiologic states which predispose certain individuals or groups to diseases. Noe *et al*. (2010) in a study on parasitic infection of brown trout (*Salmo trutta*) found Ich more frequently in precocious mature pre-spawning males which were associated with an increase in prevalence and severity of infestation with ectoparasites. Reproductive stress may also be a factor in the apparent variation in susceptibility to infection. Woo *(*1995) proposed that there are physiological races of *I. multifiliis* and suggested that the physiological races are related to the temperature tolerance of *I. multifiliis* that infect cold water (7.2 - 10.6°C) fishes such as salmon and others that infect warm water (12.8 - 16.1°C) water tropical fishes. Fishes with wide ranges of temperature tolerance such as carp and catfish may be susceptible to both cold and warm parasites.

1.2.2. Outbreaks of Ich infection

1.2.2.1 Seasonal fluctuation in infection

Outbreak of *I. multifiliis* occurs when conditions are favourable for rapid multiplication of the parasite. These include a suitable environment and susceptible fishes. According to Noe *et al*. (2010) fish density is not a constraint on the establishment of infection. However, there does appear to be a requirement for some minimum number of fish before an epizootic population of fishes.

A critical condition for an outbreak is water temperature. The duration of the development cycle of *I. multifiliis* is significantly influenced by temperature. Generally, as the temperature rises (up to 25 - 28°C), parasite activity increases and the life cycle is completed in a shorter time than at lower temperatures. In addition, the number of tomites in each cyst varies with the temperature of the water.

Stress can bring about an outbreak in a fish population. Stress is a complex physiologic reaction that causes the release of steroids from the adrenal glands which in turn decreases the immune

function of the host. A wide variety of factors can induce stress in fishes. These include crowding, low dissolved oxygen, chemical pollutants in the water, high temperature and spawning activities.

Ichthyophthiriasis is most likely to occur when fish are stressed and the water temperature is relatively warm. Epizootics occur in aquarium raised fishes when optimum conditions for parasite development and rapid multiplication are present. The parasite reproduces when fishes are stressed and water temperature is relatively warm. Epizootics occur in aquarium-raised fishes when optimum conditions for parasite development and rapid multiplication are present. The parasite reproduces when fish are under stress from low oxygen and/or crowded conditions. In temperate zones outbreaks occur in the spring as the water warms and when fishes are spawning (Woo, 1995). This is very apparent in reports of disease in sub-arctic areas. *I. multifiliis* epizootics in Finland occurred when water temperature was above 14°C and stopped as soon as the temperature fell.

The cyclic nature of outbreaks is also influenced by development of immunity in the fish population (Woo, 1995). It is well documented by (Woo, 1995) that fish infected with *I. multifiliis* develop protective immunity. Epizootics occur when there is a sufficiently large population of susceptible fishes. As the infection progresses, the highly susceptible fishes die while those that are most resistant develop immunity. With time, the majority of the surviving fish will be immuned and these conditions develop, the epizootics wanes and losses stop. The surviving population of fish breeds and with time, the level of disease resistance decreases; this will initiate another epizootic under the right condition.

1.2.2.2 Parasite Survival

The ciliate is an obligate parasite and as such requires susceptible hosts to propagate. There is no evidence of a resistant stage to ensure survival during environmental changes or in the absence of fishes hence, it must maintain a low level infection in a population.

Temperature is an important factor in the persistence of an infection in a fish population. When the water temperature is low, the developmental period of the parasite is increased. The growth period of trophonts on the fish varies from 1 week at 20°C to 20 days at 7°C (Woo, 1995). Also, it can occur at temperature as low as 2 - 4°C. (Bauer, 1970) found no density ï dependent constraints on the establishment of Ich infections in naïve hosts. There was, however, significant variation in the number of parasites found on each fish.

These differences were attributed to such factors as the amount of mucus produced by individual fishes, nutritional status, and stress. In a subsequent study, (Bauer, 1970) examined the role of host death on the reproductive potential of *I. multifiliis* in a population of susceptible Fishes. He concluded that the low prevalence between epizootics was consistent with the idea that *I. multifliis* and fish population are regulated by parasite induced host mortality.

1.2.3. Parasite morphology and life cycle

I. multifiliis (Ich) is holotrichous histophagous ciliates. It possesses a large sausage or horse-shoe shaped macronucleus and at least one small round micronucleus (Woo, 1995). The micronuclei of ciliate are transcriptionally inactive and play a role in genetic exchange. While micronuclear exchange (autogamy) occurs in some ciliates, others reproduce indefinitely without it. It is not known whether *I. multifiliis* undergoes autogamy. Experience indicates that laboratory isolates maintained by serial passage/or infection cycles on fishes gradually lose their infectivity and appear to become senescent. When this occurs, the infection can usually be ñregeneratedò by introducing a new isolate. It is not known whether the old population is replaced or whether the two isolates undergo a process of genetic mixing.

I. multifiliis cyclically transforms between an obligate fish-associated trophont and a free-living reproductive stage, the tomont. The theront is the infective stage of the parasite. The theront is pyriform to fusiform in shape with a tapered posterior end. It is about 30 x 50µm in size, but this varies greatly depending on the initial size of the tomont. The theront is completely covered with cilia each of which measures approximately 5.0 µm in length by 0.2 in diameter. A longer cilium, two to three times in length of the others, protrudes from the posterior end of the cell.

The theront swims rapidly, frequently changing direction until it makes contact with a host. The parasite is attracted towards light and is positively chemotactic to light. Theronts swim for approximately four days but they may not be infective for the entire period (Fig. 1). The theronts get attached to the surface epithelium of the skin and gills and penetrate within five minutes to the basal layer. Secretory mucocysts concentrated at the apical end of the theront are discharged as it approaches the fish and the adherent theronts appear to stick to epithelia by means of their cilia which supported the report of Kozel (1986) who suggested that theronts attach by means of specialized thymatic cilia.

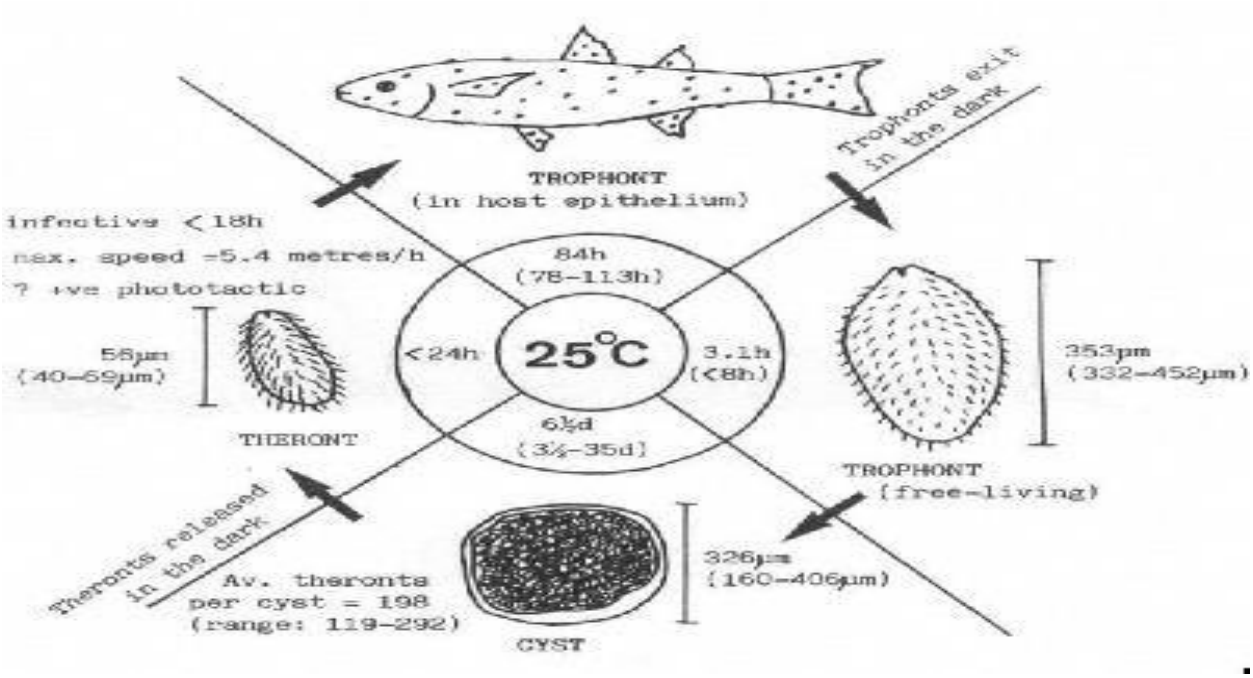

Fig. 1. Life cycle of *Ichthyophthirius multifiliis*

Source (Abowei, 2011)

The most important structure associated with penetration is the apical perforatorium. It consist of a group of apical ectoplasmic ridges, each arising between ciliary rows, that converge to form a 1.5 to 2 µm protrusion at the top of the theront. The parasite uses this structure to physically force its way through the epithelial layers. Small primitive buccal apparatus of the theront changes in the trophont to a vestibula cavity that is large enough to ingest relatively large cell debris. The theront virtually eats its way into the epithelium that differentiates immediately upon entering the host. In another study, Woo (1995) showed an electron micrograph depicting a young trophont (40min after infection) in gill epithelium which confirmed that the theront develops a functional buccal apparatus soon after it enters the host. However, since a cytostome is not visible in Ewingôs micrograph (Woo, 1995), it was hard to discern whether the putative buccal apparatus was real or merely a fold in the membrane.

The organelle of Lieberkuhn, also referred to as the ówatch glass organelleô, lies in the oral region of the theront. It is a dense, ovoid, non membrane-face the oral cavity. The function of this organelle is a mystery although it could possibly be involved in phototaxis. It exists only in the theront stage and disappears soon after the parasite colonizes a fish.

The theront penetrates the basal layer of the epidermis and undergoes differentiation and growth. Once the vestibular apparatus becomes functional, the trophont increases dramatically in size. The digestive cycle of the parasite has been divided into three main stages based on the ultrastructure of the food vacuoles. The growth rate is positively correlated to increase in the ambient temperature. The parasite rotates and moves in an amoeboid fashion using the perforatorium to scrape cells and cell debris from the edge of the lesion, which it then ingests. The ciliateôs surface is continually thrown into folds as it rotates and moves in the epithelial tissue. The large horseshoe-shaped macronucleus is readily apparent under a light microscope. The parasite continues to grow by adding ciliary rows as well as membrane and cytoplasmic organelles (mucocysts). The contractile vacuole of the theront multiplies to several hundreds in the trophont. The trophont creates a tissue space in the epithelial layers. The parasite within this vesicle appears as a white spot about one millimeter in diameter. Large number of these are easily visible, hence the common name ñwhite spot diseaseô. Occasionally several parasites occur within the same vesicle. Most researchers have attributed these to theront penetrating within the same path or co migration within the epithelium. Bushra (2010) however, calculated that the number of trophonts within a particular section of epithelium increased during infection,

and postulated that parasites multiply within vesicles. Definitive proof of cell multiplication on the fish requires direct demonstration of trophonts in the process of dividing; this has not yet been observed.

The duration of infection is variable and depends on different factors. These include temperature, fish species, physiological state of the host, and body region in which the parasite resides. If an infected fish dies, the trophont leaves presumably in response to change in oxygen tension and the pH of the tissues. Before the parasite can survive, however, it must first attain a critical size and stage of differentiation. Woo (1995) reported that parasites of less than 95µm were not viable if removed from a fish. Later, and in agreement with Woo (1995), survival ranged between 85 and 104 µm. The time required to reach these sizes is primarily a function of the water temperature and the size of the parasite when it initially infected the fish. Moreso,trophonts attained these sizes within two days at 27^{o}C (with a calculated growth rate of 5.9% per hour), and three days at 22^{o}C (with a growth rate of 8.3% per hour although, and close to 100% of the trophonts that left after two days at 21^{o}C and 24^{o}C were able to survive. He also reported that parasites associated with the fish for longer periods (four days as opposed to three) were able to exit the epithelium more quickly when the host died. When mature, a trophont can exit the epithelium, secrete a cyst, divide, and produce active, infective theronts. Elser, (1999) proposed that before the parasite can survive outside the host, its contractile vacuole must develop so that it can respond to the osmotic changes that occur as the trophont leaves the epithelium.

Dickerson *et al*. (1998) injected theronts into the abdominal cavities of channel catfish (*Ictalurus punctatus*) where they grew for up to three weeks. The trophonts eventually died without secreting a cyst or undergoing divisions, and the dead parasites became enclosed within granulomatous tissue. The trophont becomes a tomont as soon as it ceases to feed and extricates itself from the epithelium. This is the reproductive stage of the organism. The parasite usually left the fish at the stage, but sometimes it remains superficially attached to the surface mucus. Secretory mucocysts of the tomont are discharged to produce a gelatinous cyst wall in which the cell undergoes multiple binary divisions. The period between leaving the fish and secreting the cyst wall is influenced by temperature. At 21 - 23° C, the tomont swims for approximately an hour before it secretes the sticky proteinaceous matrix. Tomonts when placed in water at a temperature below 10°C do not secrete a cyst wall or divide. When cooled cells are warmed to 21 - 23° C, they secrete a cyst and proceed to divide normally.

The tomonts attack virtually any substrate in the immediate aqueous environment. Occasionally, however, encysted tomonts are found free floating or in the surface mucus of moribund or dead fishes. Bushra (2010) confirmed that the cyst consists of two layers, an inner homogeneous layer with the same consistency and electron density of the material found in mucocysts, and a less dense outer layer covered with bacteria and other debris. Bushra (2010) found that the wall varied in width with the thickest side at the point of attachment to the substrate. In some instance, secretion of the cyst wall continues after the first or second division, resulting in partitioning into chambers within which the daughter cells divide. It was further observed that cyst wall could be removed following the first division without affecting the viability of dividing organism.

Shortly after the parasite secretes the cyst wall, it begins a series of palintomic divisions, which results in 200-800 tomites. The period between secretion of the cyst and the first cell division is dependent on temperature and tomont size. At 23°C, the first division usually occurs between 30 and 75min. At the same time, the vestibular buccal apparatus and the perforatorium are resorbed. The number and size of tomites varies and depend on the number of cell divisions which is correlated directly with initial size of the tomont.

Divisions are completed in 18-24 hours at 23°C. The tomont usually divides eight to nine times to produce 256 to 517 tomites. Occasionally, more divisions will occur producing more daughter cells. These develop into infective theronts. Differentiation of tomites into theronts involves the acquisition of a pyriform cell shape, the development of a rudimentary buccal apparatus, and the formation of a perforatorium. Food vacuoles are not evident in theronts, although small vacuoles with acid photophatase activity have been observed by Woo (1995). The first theronts to differentiate bore their way out of the cyst leaving holes in the wall through which the remaining organisms leave.

1.2.4. Host Parasite relationship and distribution

Ichthyophthirius multifiliis does not appear to have a predilection for any specific group of fishes, although it is believed that the organism originated as a parasite of carp. Woo (1995) indicated that there was a linear relationship between the number of theronts to which a host was exposed and the resultant parasite burden. Epizootics of *I. multifiliis* appear to occur uniformly in populations of male and female fishes. However, there are reports (Woo, 1995, Bushra, 2010 and Qiuzong *et al*, 2013) where infections occurred predominantly in one sex. Male guppies (*Lebistes reticulates*) were seen to be more severely infected than females. Similarly, in brown

trout (*Salmo trutta*) mature males were more frequently infected than females, with the most severe infections occurring on precociously mature pre-spawning males. In contrast, in an epizootic in Lake Titicaca, the majority of the dead and infected fish was gravid or spent female killifish (*Orestias agassii*) (Wurtsbaugh and Tapia, 1997). Infection may not be a function of predilection of the parasite for either sex, but rather that infection occurred on fishes under the greatest stress. Reproductive activity is a significant stress on fishes.

Fishes exposed to *I. multifiliis* develop protective immunity; hence survivors of an epizootic are resistant to subsequent infection. Therefore, in native populations, young fishes might be more susceptible to infection than older individuals if the latter were previously exposed to the parasite. In naïve feral fish populations, all ages appear to be equally susceptible to infection. Except in very severe infection, *Ichthyophthirius multifiliis* is not uniformly distributed on the body of the host fish. The parasite occurs most frequently on the dorsal surface, particularly the head and fins.

An extremely important site of infection is the gills. The development of trophonts within the gill epithelium is a major factor in the lethal effects of infection. Large numbers of theronts come in contact with the gill epithelium because large quantities of water pass over these surfaces. Infection is the result of random encounters between the fish and infective theront contact. In addition, gills do not have the same degree of protection against parasite infection as the skin. The surface of the fish is covered with a mucous layer that is part of the defense against parasites. In unstressed fishes, there is very little mucus covering the secondary lamella of the gills, thus, gills appear to be more susceptible to infection than body surfaces (Woo, 1995).

1.2.5. Genetic susceptibility

Woo, (1995) noted that in experimental *Ichthyophthirius multifiliis* infections, only a portion of the infecting theront population developed into trophonts. He concluded that this variability was a result of host factors which might be genetically controlled. One majority host resistance factor is the production of surface mucus. Woo (1995) demonstrated significant variation in susceptibility to infection among fish species and hybrids and found that by removing effects due to other variables (such as time of infection and temperature) heterotis (hybrid vigour) contributes to resistance to *I. multifiliis*.

I. multifiliis is believed to have originated as a parasite of carp (Hoffman, 1989). The long host-parasite association may have led to the development of strains of carp that are more

resistant to infection. Preliminary genetic studies using scale patterns as genetic markers suggested that strains of carp vary in their resistance to *I. multifiliis* (Clayton and Price, 1994).

1.2.6. Behavioural modifications

In the early stages of disease, fishes congregate near water intake to reduce contact with free-swimming theronts (Woo, 1995). Fishes also flash or rub their bodies against objects in reaction to skin and gill irritation caused by the theronts. Fishes swim more rapidly than normal and often leap out of the water. As the disease progresses they become less active and congregate at the bottom of ponds or aquaria. Fishes also lie near the edges of ponds moving their gill opercula rapidly in an attempt to obtain more oxygen. This is related to gill damage caused by the parasite. With very heavy infections, fishes become lethargic and stop feeding.

1.2.7. Gross pathology

In less severe *I. multifiliis* infections, the only detectable pathologic changes may be the presence of a few white spots on the surface of the fishes. In more severe cases, there are usually large numbers of spots on the skin. Occasionally, however, *I. multifiliis* only infects the gills and hence there are no obvious gross lesions on the body surface (Brown and Gratzek, 1980). Ulcers develop in the skin of heavily infected fishes and are often the sites of secondary bacterial or fungal infections. The fin becomes frayed due to loss of tissue between the fin rays (Hines and Spira, 2004). Mucus production is increased. Heavily infected carp have a thick lumpy covering of surface mucus (Hines and Spira, 2004). Increased production of mucus is not a unique response to *I. multifiliis*, however, as it occurs on most fishes that are exposed to irritants or skin parasites. Infected fishes have enlarged spleens and kidneys and pale mottled livers. Also transudate fluids are found in the peritoneal cavities. It is not clear that the changes in the viscera are directly due to the activity of the parasites. These may be secondary effects caused by opportunistic bacterial or fungal infections and/or anorexia of the fishes in the later stages of the infection.

1.2.8. Clinical signs

The common clinical signs of Ichthyophthiriasis are the characteristic white spots. Each spot represents a development trophont within an epithelial capsule or vessel. Visible spots develop several days after initial attachment of theronts. In cases where the infection is restricted to the gills these are not visible.

One of the first physiologic responses to infection is an increase in surface mucus production. Skin penetration by theronts stimulates expanded numbers of mucus-secreting cells

in the epidermis. These multiply not only in areas around the parasite, but throughout the epidermis. In severe infections, mucus may stream off of the posterior edge of the fins and tail.

A careful observer can detect infection before the development of surface tension by noting changes in fish behavior. Initially, infected fishes swim more rapidly and rub themselves against objects, and the disease progresses, the fish surface and gasp for oxygen and become increasingly lethargic and eventually cease feeding.

In severe infections there is erosion of the epithelia leading to ulcer formation and exposure of the deeper tissues to bacterial and fungal invasion. A common cause of secondary invasions is the fungus, *Saprolegnia* spp., which appears as tufts of ófuzzô on the skin.

The clinical signs of a *C. irritans* infection are similar to those seen in ichthyopthiriosis. Fishes develop small white cysts on their body surfaces (Yoshinaga and Dickerson, 1994). The lesions are often numerous minute grayish vesicles rather than the lager white spots associated with Ich infections. *C.irritans* infection frequently involves the eyes which lead to corneal clouding and blindness. Marine fishes infected with *C.irritans* produce excessive amounts of surface mucus.

1.2.9. Histopathology

The parasite load is the major factor contributing to diverse tissue damage. The nature and severity of histopathologic changes seen in *I. multifiliis* infections vary greatly. This variation is influenced by such host factors as stress and nutritional status. However, the parasite load is the major factor contributing to the diverse tissue changes. In general, mild infections elicit minor cellular reactions. This minor host reaction suggests that *I. multifiliis* is, in evolutionary terms, a long-standing parasite of fishes and that, as a result, the host and parasite have a relatively benign relationship. The extensive histopathologic changes reported to occur in *I. multifiliis* infections are only seen in severe epizootics or in experimental infections with large numbers of parasites (Ventura and Paperna, 1985).

1.2.9.1. Histopathology of the skin resulting from mild infections

In primary *I. multifiliis* infections (that is, the first contact of the parasite with the fish) there is little reaction to the penetrating theronts. After 40 hours, most trophonts will have penetrated the epithelial layers and be located next to the basement membrane. The cells between the parasite and the basement membrane become hydropic, vacuolated or necrotic with pyknotic

nuclei. The growing trophont gradually lifts and displaces the epithelial cell layers until it lies within an epithelial capsule that extends above the skin surface. The epithelial layer overlying the trophont expands to cover the parasite during this growth stage. The epithelium retains its architecture of differentiated cell populations. Cell damage is observed only in the cellular layers in direct contact with the developing trophont. Host cell debris can be observed in the food vacuoles of the trophont and in the spaces in the epithelial capsule around the parasite. There is evidence of haemorrhage occurring in the skin as a result of parasite invasion. Large pale-staining alarm substance cells have been observed in the areas of the developing trophont. In mild infections, only a few leukocytes are seen in the epithelium.

1.2.9.2. Histopathology of the skin resulting from heavy infections

In heavy infections a much greater inflammatory reaction occurs in the skin. Penetration by numerous theronts leads to increased epithelial cell hyperplasia. This cellular proliferation could be a defence mechanism. In addition to hyperplasia, there is a generalized increase in the number of mucous cells in the skin. The epithelium in heavily infected fishes may be up to four times its normal thickness due to proliferation of epithelial and mucous cells.

Extensive cell necrosis and histolysis occur around trophonts developing in the hyperplastic epithatics leading to oedema and an increased epithelial infiltration of neutrophils, eosinophils and lymphocytes. The outermost epithelial cells degenerate and slough off, eventually exposing the underlying basement membrane. It was suggested by Woo (1995) that the extensive cellular reaction observed in the skin of fishes heavily infected with *I. multifiliis* is a hypersensitivity reaction. In severe infections, there was a marked drop in serum Na^+ and Mg^{2+} levels and a rise in serum K^+ levels. Blood urea-ammonia levels also increased during the course of the infection.

1.2.10. Antigens of *Ichthyophthirus multifiliis*

It was observed as early as the beginning of the twentieth century that fishes which survive mild outbreaks of *Ichthyophthirius* become solidly immune to reinfection (Hines and Spira, 1974; Butcher, 1993; Bauer, 1993; Beckert, and Allison, 1994). Despite the fact that acquired immunity against Ich is well recognized, the mechanism of the response and the parasite antigens that elicit it remain largely unknown. Nevertheless, the fact that fishes develop

such a strong immunity against infection makes it theoretically possible to create protective vaccines for the control and prevention of disease outbreaks.

1.2.10.1. The use of controlled infections for vaccination

A number of researchers have attempted to vaccinate fishes against Ichthyophthirius using controlled infections. Goldfish (*Carassius auratus*) was immunized by exposing them to 0.5 and 1.0 LD_{50} doses of infective theronts. An LD_{50} dose was 108 theronts per ml of water. Immunized fish were resistant to lethal challenges of 8 to 10 LD_{50} doses. Areerat (1974) vaccinated channel catfish (*Ictalurus punctatus*) by exposing them to 20 or 40 encysted tomonts and treating the mortality than non-immunized fishes when challenged with 20 tomonts per fish. Hines and Spira (1974) immunized mirror carp (*Cyprinus carpio*) by exposing them to sub-lethal doses of tomonts. In this study, fishes recovered naturally without treatment and remained free of parasites when challenged with large numbers of tomonts (500 per fish). One hundred percent of fishes died when exposed to tomonts or infected fish in the same system.

1.2.10.2. Live Versus Killed Vaccines

In general, infection with live parasites produces a more substantial and prolonged protective immune response than vaccination with killed organisms. This is usually attributed to an increase in parasite mass as a result of growth and reproduction on the host. In addition, excretory or secretory products released by the live parasite during infection also stimulate the hostôs immune response.

Live vaccines usually consist of either a carefully controlled infection with a fully virulent parasite (such as those for Ichthyophthirius described above), or an infection with a less virulent or attenuated strain. To date, no attenuated strain of Ich has been described and the use of the virulent organism for vaccination carries the risk of inducing high mortalities in the population one is attempting to immunize. Ich cannot be easily propagated which also makes its use as a live vaccine impractical. The approach has therefore been to immunize fishes against *Ichthyophthirius* using killed parasite preparations. Goldfish injected intraperitioneally with freeze-thawed theronts, had fewer trophonts on their body surface compared to non-immunized control fish. Protection was induced after injecting channel catfish intraperitoneally with killed, trophont mixed with Freundôs complete adjuvant (Woo, 1995).

1.2.11. Prevention and control of the parasites

Disease control and prevention in an intensive fish production operation depend upon an integrated management programme. The basic elements are:

i. Prevention of exposure of fishes to the parasites;
ii. Prompt identification of the disease if it occurs; and
iii. Treatment of infected fishes and immunization (if possible).

Prevention of disease is always more cost effective than treatment.

I. multifiliis and *C. irritans* infections are usually introduced into fish populations by the addition of new infected fishes. In freshwater fish culture, no new fishes should be introduced into a facility unless they have been in quarantine for 2 to 3 weeks (Brown and Grazek, 1990). If possible, the quarantined fishes should be held in moderately warm water (24^{o}C). This is the optimal temperature for the development of *I. multifiliis* and in 2 to 3 weeks the parasite would have gone through several infection cycles. Thus, if the fishes were carrying low levels of infection, there would be ample time for it to become apparent. The water from the quarantine facility (pond, raceway, aquarium, etc) should not be circulated into any other fish-holding facility. If an Ich infection develops during the quarantine, the fishes should be treated. Treatments that control the free-swimming theronts break the cycle of infection by prevention. In addition, it is also possible to treat trophonts that are on the fishes (see chemotherapy section, below).

The ideal method to prevent infection of fishes with *I. multifiliis* is immunization. Prevention of disease is always preferred to treatment. At the present time, there are no practical, commercially available vaccines for use against Ich. A variety of components of *I. multifiliis* have been used to experimentally induce immunity in fishes. However, because *I. multifiliis* is an obligate parasite and can only be collected from live fishes, large scale production of antigenic material for vaccines is extremely difficult. It appeared that this obstacle had been overcome when Goven *et al.* (1989) reported that fishes could be immunized against *I. multifiliis* using the easily cultured ciliate *Tetrahymena pyriformis*. However, other work has found *Tetrahymena* ineffective as a protective antigen against Ich (Dickerson *et al*., 1998). They attempted to find antigens of *I. multifiliis* which induce protective immunity. Immobilization antigens have been identified as putative immunogens, and their genes have been cloned for subsequent expression in suitable vectors (Clark *et al.,* 1998). Thus, even though there is no vaccine currently available

for the immunization of fish against Ich, there is hope that one will be available in the future through the use of biotechnology.

1.2.12. Physical treatment without the use of chemicals

The objective for treatment of a disease is to break the cycle of infection. In *I. multifiliis* infection, the most vulnerable stage is the free-swimming theront. The trophont is located deep in the epidermis of the fish and is protected from most agents that are added to the water.

One simple treatment is dilution or removal of the theronts in the water. The repeated transfer of fishes to different aquaria is effective. Daily transfer for 5-7 days is usually sufficient to break the cycle of infection (Brown and Gratzek, 1998). In large fish culture operations dilution is used if the fishes can be placed in a raceway or shallow pond. A rapid flow of water is then maintained for a week to dilute out the theronts.

In closed fish culture systems where the water is recycled, Ich infections can be controlled by exposing the water to ultraviolet light. The recycled water is passed through an ultraviolet light sterilizer and exposed to 91,900µW s^{-1} cm^{-2} before being cycled back into the aquaria containing fish (Gratzek *et al.,* 1983). However, a flow rate increase (>85cm/min with a water turnover rate of >2.1/hr) was shown to greatly reduce the severity of *I. multifiliis* infections, arguably by flushing the parasite away from the rearing systems.

Theronts are killed at high temperatures (29 ï 30^{o}C). Maintenance of fishes at these temperatures for a week will eliminate infection (Brown and Gratzek, 1998). The water temperature should be raised gradually to allow the fishes to acclimate to the change. This treatment is often combined with dilution. The use of elevated temperatures to control Ich infection is unsuitable for cold water fishes. Increasing the water temperature to 32^{0}C for a period of 5 days has proven to be effective in eliminating *I. multifiliis* infection in tropical fish species (Brown and Gratzek, 1998). Finally, brushing the bottom of culture tank systems has been demonstrated to be an efficient method to remove *I. multifiliis* stages. The afromentioned methods have limited practical application on large scale farm systems where the number of infected fish can reach thousands particularly over the spring summer periods when infection level increase. The intensive production of farmed fish is often concomitant with a limited availability of water and tank space and reduction of labour intensive management practices.

1.2.13. Chemotherapy

A variety of chemicals have been used in treating *I. multifiliis.* None of the treatment is uniformly successful. Sodium chloride was one of the first to be used. In general, salt treatment does not rapidly control the infection and often does not completely remove the parasite from the environment. The use of salt may have an additional benefit. Fishes infected with *I. multifiliis* have decreased serum sodium and magnesium (Woo, 1995). The sodium in the water may help fishes maintain osmotic balance, thus reducing stress. Reduction of stress increases the fishôs defense reactions.

Formalin is often used to control infections. Fishes in aquaria are treated with 25ppm (1ml formalin to 37.5L water) of formalin on alternate days until the infection is cleared. Usually the water is changed on days between treatments (Brown and Gratzek, 1990). Pond fishes are treated with 15 to 25ppm. Bath treatments are also used; fishes are treated with 160 ï 250 ppm formalin for 1 hour daily until mortality stops (Brown and Gratzek, 1990). However, formaldehyde was reclassified as ócarcinogenicô to humansô in 2004 and it is expected that its application will not be permitted to treat fish in the near future. Rainbow trout exposed to formaldehyde concentrations of 200-300 ppm for 1hr showed a significant reduction in mucus production making the fish more susceptible to secondary infection by fungi and bacteria. Other treatments used to treat the free swimming stages of *I. multifiliis* amongs others; copper sulphate, especially in channel catfish cultured in ponds in USA (Schlenk *et al,* 1998; Tieman and Goodwin, 2001) sodium percarbonate (Buchman *et al*, 2003), and humic acid (Noe *et al*, 2010). Only few chemicals are available to disrupt the trophont development, the main one being Toltrazuril (Rzgar and Buchman, 2013). Following the ban of malachite green, no chemical treatments effective against all stages of *I. multifiliis* (on and off the fish) have been identified or licenced. Repeated treatments are therefore required, which have an inherent labour cost and compromise fish homeostasis and growth performance. The chemotherapeutic strategies presently available are not satisfactory and in the case of formaldehyde, not sustainable in the longer term. Hydrogen peroxide is a powerful oxidizer which has been used under field conditions to control *I. multifiliis*. High doses, however, can cause gill damage leading to fish mortality (especially at high temperature). Its use in vitro tests against free living stages of *I. multifiliis*, however, were disappointing. Metronidazole has been shown to be very successful at reducing the number of trophonts on infected fish when incorporated into fish feed diet (Tojo-Rodriguez and Santamarina-Fernandez, 2001). This compound, however, is listed as ñpossibly

carcinogenic to humansò by the WHO and is currently banned by EU and USA for use in animal feed and in USA specifically for animals destined for human consumption. Dead fishes should be removed as soon as they are found because trophonts begin to drop off and encyst almost immediately. Aquaria should be scrubbed and water will dislodge and remove developing cyst and theronts from the aquaria. In raceways where the water flow can be increased, the bottoms should be swept daily. This will dislodge developing cyst and many will bury some cysts while others attached to the suspended bottom material can be flushed out by increasing the water flow (Brown and Gratzek, 1990).

Aquaria, ponds and raceways should be drained, cleaned and allowed to dry after an outbreak. Drying kills the parasite. The bottom of a dried pond should be treated with lime. If ponds cannot be drained completely, the residual water should be treated with a disinfectant such as calcium hypochlorite (Brown and Gratzek, 1990).

1.2.14. The emergence of natural extractss

Some new and alternative treatments include the utilization of plant extractss such as those from garlic (*Allium sativum*), which showed promising results when tested in vitro (Buchmann *et al*, 2003). However, when incorporated in-feed and tested *in vivo*, this extracts did not manage to significantly reduce infection levels when compared to the control group. Other Natural products such as those from *Carica papaya*, and the Velvet beans *Mucuna pruriens* were successful when tested *in vitro* and *in vivo* against tomonts and trophonts (Ekanem *et al,* 2004). Gholiper *et al* (2012) reported the influence of mother worth (*Matricaria chariomilla*) extracts on the treatment of Ich parasite which successfully treated the snail fin molly (*Poecilia latipinna*) within 5 days. *Zingiber officinale* (Ginger) has been used to eradicate protozoan parasite from *C. gariepinus*. The herbal control with ginger was safe and effective to treat the ectoparasite protozoan; Trichodina and *Epistylis spp* at 20mg/L. However, anti-ich efficacy of pentagallylglucose, a compound extractsed from the plant, *Galla chinnensis* was found to kill all theronts at concentration of 2.5mg/L-20mg/L during 5-6-233.9min and terminate reproduction of tomonts at 40mg/L (Qizhong *et al,* 2013). Moreso, *Azadirachtha indica* traditionally known as the ñvillage pharmacyò or ñvillage dispensaryò in India (Biswas *et al,* 2002) was used to eliminate Argulus and protect the host from ectoparasite but it was also reported to be a strong natural insecticide (Saurav *et al*, 2012). Chanagin *et al* (2005) reported the efficacy of *Allium sativum* and India Almond (*Terminalis catappa*) as optional chemical to treat ectoparasites, Trichodina. Madsen *et al* (2000) reported that raw and squeezed garlic (*Allium sativum*) at 200mg/L had potential to

treat trichodiniasis in eel. There is therefore considerable potential for the use of such natural products to control *I. multifiliis* infection. However, *in vivo* trials carried out under field trials condition are a critical requirement prior wider deployment of such treatments. The use of probiotics (*Aeromonas sobria*) has proven to be very effective at reducing infections in medicated fish (Pieters *et al*, 2008). Recent research by Yao *et al* (2010) using *Macleays cordata* extracts has showed high efficacy in *in vitro* trials against tomonts and an important trophont reduction(75 - 97%) when administered in vivo at low concentrations (0.6 - 0.9mg/L) for 48h.

1.2.15. *Moringa oleifera*

Many parts of the moringa are edible. Regional uses of the moringa as food vary widely, and include:

- The immature seed pods, called "drumsticks", popular in Asia and Africa.
- Leaves, particularly in the Cambodia, Philippines, South India and Africa.
- Mature seeds
- Oil pressed from the mature seeds
- Roots

The leaves are the most nutritious part of the plant, being a significant source of vitamin B_6, vitamin C, provitamin A as beta-carotene, magnesium and protein, among other nutrients reported by (Udupa, 1994) when compared with common foods particularly high in certain nutrients, fresh moringa leaves are considerable sources of these same nutrients.

Some of the calcium in moringa leaves is bound as crystals of calcium oxalate which may inhibit calcium availability to the body. It is not clear whether the calculation of the reported amount of calcium in moringa leaves includes such non-bioavailable calcium.

Fig. 2. *Moringa oleifera* leaves

The leaves are cooked and used like spinach. In addition to being used fresh as a substitute for spinach, its leaves are commonly dried and crushed into a powder used in soups and sauces. It is important to remember that like most plants heating moringa above 60^{o}C (140 degrees Fahrenheit) will destroy some of the nutritional value.

The moringa root bark is considered bitter, acrid, thermogenic, digestive, carminative, anthelmintic, constipating, anti-inflammatory, emmenagogue, diuretic, ophthalmic, expectorant and stimulant. It is used in treating dyspepsia, anorexia, verminosis, diarrhoea, colic, flatulence, paralysis, inflammations, amenorrhoea, dysmenorrhoea, fever, and strangury, vesical and renal calculi. It is used in cough, asthma, bronchitis, pectoral diseases, splenomegaly, epilepsy and cardiopathy.

The root bark and bark of moringa are used for poor circulation, to increase appetite and stimulate digestive system and also taken as a tonic. Root bark is ground and mixed with salt to form a poultice which is administered for rheumatism and muscular pains, and the bark is considered as antiscorbic. Moringa leaves possess anti-inflammatory, anodyne, ophthalmic, anthelmintic properties and they are rich in Vitamin A, C and D. They are useful in scurvy, wounds, tumors, inflammations and helminthasis, act as a general tonic for infants. The leaf of moringa is seven times vitamin C of the orange, 4 times calcium of the milk, 4 times vitamin A of the carrots, 3 times potassium of bananas and 2 times protein of the yoghurt. (All parts of moringa are available for wholesale) (Udupa, 1994).

Moringa seeds are used as a water-soluble extracts in suspension, leading to the effective natural clarification agent for highly turbid and untreated pathogenic surface water. Such seeds of moringa make water drinkable as the turbidity and bacteria are reduced, and they are also used in preparation of cakes, and many other bites as a nutritive. Moringa seed oil is beneficial for hair problems and is widely used in preparing cosmetics. Moringa seeds are considered acrid, bitter, anodyne, anti-inflammatory, purgative, antipyretic, and ophthalmic. They are also used in treatment of neuralgia, inflammations, intermittent fevers and opthalmopathy**.** No work has been reported on the use of *Moringa oleifera* in treatment of fish disease.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Collection and Disinfection of Experimental Fish

A total number of 800 apparently healthy parasite free post-juvenile catfish (*Clarias gariepinus*) of mean weight 102.41±11.51g was obtained from Freedom Fishery Nsukka, Enugu State and was transported to the Department of Zoology and Environmental Biology, wet lab, University of Nigeria, Nsukka. Prior to acclimatization the fish were disinfected with 0.5% potassium permanganate in 5L of water in a quarantine tank to remove any possible external parasites. The fish were acclimatized for two weeks and also kept in a well aerated chlorine free tap water throughout the experimental periods at a temperature of 22.3^0C - 24.2^0C, p^H 6.7 ï 7.2 and dissolved oxygen 5.7 mg/L ï 6.9 mg/L. The fish were fed daily with commercial feed (Copens). The water quality maintained daily with a standard test kits.

2.2. Collection and Preparation of Experimental Aqueous Extracts of *Moringa oleifera*

Fresh leaves of *Moringa oleifera* were collected from Nsukka Environment and identified using é é in the herbarium unit of the Department of Botany, University of Nigeria, Nsukka. Thereafter, the leaves were dried under room temperature in a wet laboratory. The dried leaves were ground into powdery form and sieved using 0.3µ musilin cloth. The powdered herbs were concentrated in water, shaken vigorously and left for 24h after which the solution was filtered with musilin cloth and the filtrate concentrated to dryness using rotary evaporator. The extracts was weighed and then sent for phytochemical analysis.

The LC_{50} of the *Moringa oleifera* aqueous extracts was administered using the following concentrations (2800mg/L, 6400mg/L and 8300mg/L) of the extracts after the range finding test.

The result helped to determine the safe range of the extracts, for no mortality occurred throughout the trial test irrespective of increased concentrations.

2.3. Experimental Design

The experiment was designed as a factorial experiment in complete randomized design.

Seven groups (A-G) of post juvenile *Clarias gariepinus*, were used for the study. However, the experiment was conducted in three different phases of treatments thus:

Phase 1. Dip (I h/or 60 mins)

Phase 2. Short-term treatments (24 - 96 h)

Phase 3. Prolonged term treatments (5 - 15 days).

At each stage of each experiment, the fish were maintained in experimental groups (A-G) with three replicates per group. The varied concentrations of aqeous extracts of *Moringa oleifera* leaves extracts are shown below based on concentrations reduction factor:

1. Concentrations selected for the dip treatments (60minutes) were:

Group A: Non infested (Normal control)
Group B: Infested not treated (Negative control)
Group C: Infested treated with standard drug (Fish cure) (Positive control)
Group D: Infested treated with 1,500mg/L aqueous extracts of *Moringa oleifera* leaves
Group E: Infested treated with 2,500mg/L aqueous extracts of *Moringa oleifera* leaves
Group F: Infested treated with 3,500mg/L aqueous extracts of *Moringa oleifera* leaves
Group G: Infested treated with 4,500mg/L aqueous extracts of *Moringa oleifera* leaves

2. Concentrations selected for the short-term treatments (24 - 96 h) were:

Group A: Non infested (Normal control)
Group B: Infested not treated (Negative control)
Group C: Infested treated with standard drug (Fish cure) (Positive control)
Group D: Infested treated with 150mg/L aqueous extracts of *Moringa oleifera* leaves
Group E: Infested treated with 250mg/L aqueous extracts of *Moringa oleifera* leaves

Group F: Infested treated with 350mg/L aqueous leaf extracts of *Moringa oleifera*
Group G: Infested treated with 450mg/L aqueous leaf extracts of *Moringa oleifera*

3. Concentrations selected for the prolonged-term treatments (5 - 15 days) were:

Group A: Non infested (Normal control)
Group B: Infested not treated (Negative control)
Group C: Infested treated with standard drug (Fish cure) (Positive control)
Group D: Infested treated with 15mg/L aqueous extracts of *Moringa oleifera* leaves
Group E: Infested treated with 25mg/L aqueous extracts of *Moringa oleifera* leaves
Group F: Infested treated with 35mg/L aqueous extracts of *Moringa oleifera* leaves
Group G: Infested treated with 45mg/L aqueous extracts of *Moringa oleifera* leaves

During the treatment period all groups were maintained in 30L plastic tank and concentrations maintained during the treatment periods. Partial water changes were done because they were vital to the speedy recovery or well being of the fish.

Table 2: Experimental design

Treatment	Group A (control)	GroupB Infested treated with standard drug (Fish cure)	GroupC Infested not treated	GroupD moringa aqueous extracts	GroupE moringa aqueous extracts	GroupF moringa aqueous extracts	GroupG moringa aqueous extracts
No. of Fish	A1:10 A2:10 A3:10	B1:10 B2:10 B3:10	C1:10 C2:10 C3:10	D1:10 D2:10 D3:10	E1:10 E2:10 E3:10	F1:10 F2:10 F3:10	G1:10 G2:10 G3:10

2.4. Parasite Culture (Serial passage by cohabitation) Through "Natural outbreak"

All infected fish collected from Anambra River, Anambra State, as potential initiators of infection were held in isolation in 20 L volume of water in plastic aquaria prior to passage. Supply of parasites was maintained in a manner that parasitized catfish (tagged by a slit at the down caudal fin) were held in 30 litres plastic tank at 23^{o}C. Healthy fish 20 in number tagged by a slit cut at the upper caudal fin were added at every 15 - 18 days periodically to sustain the parasite culture and all dead hosts were removed from the tank within 24 h. During this period, several cultures were lost and had to be restarted. However, as modification, parasites were further cultured at 24^{o}C in the plastic tank and a substrate of soils and rocks were placed in the trough (plastic tank) with constant aeration and were able to maintain Ich parasite. If the parasites were not used immediately they were refrigerated at 5.5^{o}C so as to depress the fission rate of the parasite and to further make use of the parasite. Furthermore, the infected fish collected from the wild which were used as parasite source were allowed to yield parasite either by scrapping off the body or by submerging the infected fish into a plastic basin containing a little water. The duration of the parasitic phase was about 5 days - 2 weeks and during the period of serial passage of parasite infection and approximately 20 - 30 fish were infected at staggered interval per week. Care was taken by proper aeration of water, rule of separate nets and checking for visible manifestation of symptoms and to initiate isolation in all cases to commence immediate distribution of the infected fish for bath treatments, so as to ensure that secondary infection does not occur.

2.5. Collection of *Ichthyophthirius multifiliis* Infective Stage (The theronts)

During the cohabitation period, numerous actively swimming theronts were discovered under a binoculars microscope and were quantified by extrapolation to detect the number of viable theronts that were produced. The harvested theronts were used to infect the healthy fish (uninfected) which were randomly distributed in all the groups with 10 fish per replicate (A-G) in which B-G were infected with infective stage of theronts.

2.6. Parasite Challenge

Seven (7)Two hundred and fifty (250ml) volumetric flask were used to culture the infective theronts and a sub sample (10ml) was collected from each volumetric flask and viewed under the microscope and the theronts counted. The number obtained (2,500 theronts) was used to

extrapolate the overall number (44,000) of actively swimming infective ciliated theronts present in the 250ml volumetric flask.

Approximately 44,000 theronts were administered to each group B-G (in order to secure the same infection pressure and environmental conditions for all fish groups). The groups B-G excluding the control were challenged with isolated theronts from the cultured water samples whichwas kept for 24 ï 48 h. During the challenge test, the fish were kept in darkness and were allowed to remain for a period of 14 days at 24^{o}C. Active white spots were seen on the body of the fish within this period. In other to validate infection by Ich, blood samples and tissues were collected to ascertain the state of the fish before treatment (pre-treatment) and after treatment (Post-treatment). However, the rule of separate nets for each aquaria and each isolate of parasite was strictly adhered to in order to prevent premature infection of stocked fish and cross contamination of isolates. All aquaria were checked daily for pH, temperature and dissolved oxygen throughout the study period.

2.7. Estimation/Counting of Parasites in Infested Fish

The estimation and counting of parasites in the experimental group (B-G) was done using total of 6 fish per group before (Pre-treatment) and after (post-treatment) the experiment as follows;

2.7.1. Enumeration of parasite in gills of Ich infected fish

The fish were euthanized by brain incision using a scapel and parasites were then isolated by cutting through the operculum to obtain the gills. The excised gill was cut through the gill arch to obtain three gill archs containing the filaments and the lamellae. They were placed on a slide and three drops of water added and covered with cover slip and examined under a binocular microscope magnification X40.. The number of parasites on three randomly selected zones was counted and estimate of total number of parasites recorded.

2.7.2. Enumeration of parasites in the skin smear of infected fish

Similarly, the parasites were collected by scraping the fish body (6 fish per group) left and right sides and the mucus scraped out was transferred into a microscopic slide with a little drop of water added and viewed under a binocular microscope using high power objective lens x40.

2.8. Antiprotozoal Activity of Aqueous Extracts of *M. oleifera* Leaves on the Survival of Theronts after 24 h

Approximately 900 theronts in 1ml of culture water containing theronts were distributed in 3 replicates of 18, 250ml beakers (A - F). Different concentrations of *M. oleifera* were added in each beaker to form a final concentrations of 0.01g/100ml, 0.02g/100ml, 0.03 g/100ml, 0.05g/100ml and 0.08g/100ml and 0.0g/100ml (negative control), respectively. They were allowed to stand for 24h after which the number of dead and alive theronts were enumerated and recorded. The mortalities observed in different groups were subjected to probit analysis using Finney (1952).

2.8.1. Assessment of morphological alterations of infected theronts following antiprotozooal activity of aqueous extracts of *M. oleifera* leaves

Live or dead theronts obtained from the antiprotozooal test were enumerated with binocular microscope at the end of the pre-treatment exposure period of theronts to *Moringa oleifera* to ascertain or check any possible morphological alteration in the infective stage of the parasite (theronts). Theronts were photographed with a digital camera installed in the same microscope at x40 magnification.

2.9. Evaluation of infection Potential/or Viability of Theronts (infective stage) Following Pre-treatment with Aqueous Extracts of *M. oleifera* leaves

Theronts were exposed to 0.5g/L of *Moringa oleifera*. The theronts which survived were isolated with a plastic pipette and transferred immediately to a petri dish, counted and then 15 uninfected/disinfected fish were separated into two containers (A - B) with 5 fish per replicate to ascertain whether the theronts could still be viable in infecting the healthy Ich-free fish for 14 days. The water temperature was maintained at 22^{o}C - 25^{o}C. However, at the end of the infection cycle, each fish in each container was removed and the skin scrapped into slide with addition of 2-3 drops of water and then covered with cover slip and viewed under the binoculars microscope. The number of each stage of the parasite found (trophont or theront) as well as white spot on the body and water were noted.

2.10. Determination of Infective Prevalence and Intensity of Theronts Pre-Treated for 1h with Aqueous Extracts of *M. oleifera* leaves.

Infective prevalence was estimated using the formula:

Infective prevalence (%) $= \frac{number\ of\ infested\ catfish}{number\ of\ total\ catfish} X \frac{100}{1}$

Infective intensity (%) $= \frac{number\ of\ white\ spots\ on\ catfish}{number\ of\ infested\ catfish}$

2.11. Gross Morphology Examination

Macroscopic examination (gross morphology) of the infected fish were checked for the detection of viable lesions in the body, eye, gills and fins using hand lens and dissecting microscope while the observed changes were photomicrographed

2.12. Histopathological Examination

Tissue specimen from affected fish (skin, muscle and gills) were collected after clinical and gross examination and immediately fixed in 10% neutral buffered formalin. Dehydration was done using ascending grades of ethanol (70%, 80%, and 100%) for 1hour each. The specimen was then cleared in two changes of xylene. After mounting using soft paraffin, serial sections of 4µm thickness were done. The sections were stained using routine heamatoxylin and eosin stain and examined using binocular microscope and photomicrographed using motic camera magnification by 100X and 400X.

2.13. Determination of Proximate Composition (AOAC, 1989)

The fish samples were gutted, thoroughly washed and weighted for analysis.

2.13.1. Moisture content determination

The moisture content of the fish samples was determined by washing the glass dishes thoroughly, oven-dried, after which they were cooled inside the dessicator and each of the dishes were weighed. The fish samples were mixed and the laboratory samples were taken and the weight of dish plus weight of the fish samples were taken respectively. The fish samples were dried in the moisture oven at 80^{o}C for 2 hours and at 135^{o}C for the next 4 hours or until weight are constant. The samples were cooled in the desiccators and their dry weights pus dishes were taken. Their moisture content was calculated thus;

$$\% \text{ Moisture} = \frac{W2 - W3}{W2 - W1} X100$$

Where;

W1 = Initial weight of empty crucible

W2 = Weight of crucible + Fish sample before drying

W3 = Final weight of crucible + Fish sample after drying

2.13.2. Protein determination by kjeldahl's method

A 2g of the fish samples were weighed into 30ml Kjeldahl flask. A 15ml conc. sulphuric acid was added, after which 1g of the catalyst mixture was added. The samples were heated cautiously on digestion rack under fume hood until a greenish clear solution appeared. Thereafter, digests were cleared (about 30 minutes). They were heated for extra 30 minutes. Each digest was allowed to cool and10ml of distilled water was added to avoid cakeing. They were transferred to the Kjedahl distillation apparatus and 50ml receiver flask containing 5 ml boric acid-indicator solution was placed under the condenser apparatus so that the tip was about 2cm inside the solution. A 10ml of 40% NaOH solution was added to the digested sample in the apparatus through funnel stop cork. Distillation commenced immediately by closing the steam bypass and opening the inlet stop cork on the steam jet arm of the distillation apparatus. When distillate reached 35 ml mark on the receiver flask, distillation was stopped still closing inlet stop cork first, then opening steam by-pass. The condenser tip was rinsed with distilled water and the distillate titrated to first pink colour with 0.01M HCl and calculations of nitrogen content was done.

Vreact H_2SO_4=V tot H_2SO_4 ï V_{EX} H_2SO_4

2.13.3. Carbohydrate determination by anthrone method (Shields and Burnett, 1960)

A 2g of the fish samples were homogenized with 20ml of trichloro acetic acid (TCA) for 10 minutes. The homogenate was poured into a suitable centrifuge tube and centrifuged for 15 minutes at 800 rpm. The supernatant fluid was decanted onto an acid-washed filter paper that

was placed in a funnel, draining into a graduated cylinder. The residue was homogenized again with 20ml of TCA and centrifuged again. This was done in order to extracts up to 95% of the glycogen present. Moreso, 5ml of 95% ethanol were added to each tube with careful shaking, to effect proper mixing which was noticed by the absence of an interface. The tubes were covered with clean rubber stoppers and allowed to stand overnight at 27°C room temperature. After precipitation was complete, the tubes were centrifuged for 10 minutes at 800 rpm. The clear liquid was gently decanted from the packed glycogen and the tubes were allowed to drain in an inverted position for 5 minutes. The glycogen was dissolved by adding 2ml of distilled water and shaken to dissolve the glycogen.

The glucose standard was prepared from a stock glucose solution by dissolving 0.08g of glucose into 100 ml of distilled water. The standards were prepared by adding 0 ml, 10 ml, 20 ml, 30 ml, and 40 ml, of the stock solution which were made up to 100ml with distilled water. 1 ml of each of the standard solution and the test samples were used for the extractsion.

At this stage, 5ml of Anthrone reagent was added into the test tubes containing the standard solutions and the samples. Each tube was covered and placed in a boiling water bath for 15 minutes and cooled in a cold water bath. Their absorbances were measured at 620 nm. The concentrations of the glucose were read off from standard glucose curve plotted. Reagent blank was prepared by pipetting 1 ml of water and adding 5 ml of Anthrone reagent.

2.13.4. Fat determination using soxhlet extractsion method

A 250 ml of clean boiling flasks was dried in oven at 105^{o}C for 30 minutes, transferred into desiccators and allowed to cool. A 2g of the fish samples were weighed into labeled thimbles. Corresponding labeled cooled flasks were weighed and the flasks filled with 300ml of petroleum ether. The extractsion thimbles were plugged lightly with cotton wool. Soxhlet apparatus was assembled and allowed to reflux for 6 h. The thimbles were removed and petroleum ether was collected at the top container of the set up and drained into a container for re-use. When the flask was almost free of petroleum ether, it was removed and dried at 105ęC for 1 hour. It was transferred from the oven into a desicator and allowed to cool and then re-weighed. Calculation was made thus;

$$Percentage\ Fat = \frac{Weight\ of\ Fat}{Weight\ of\ Sample} X100$$

2.14. Haematology

2.14.1 Evaluation of packed cell volume

The infected fish blood was obtained by the severance of the live fish and collected in vaccum tubes containing EDTA anticoagulant. The haematocrit was determined by filing one capillary tube with blood from each fish and centrifuging in a microheamatocrit (Hawksley England) at 300 rpm for 5 minutes at 27° C room temperature. Soon after, the heamatocrit was read using a heamatocrit reader and reported as percentage of the whole blood (Wintrobe, 1967).

2.14.2. Evaluation of haemoglobin

The heamoglobin was determined using the cyanmetheamoglobin method (Wintrobe, 1967)

2.14.3 Evaluation of red blood cell count

The erythrocytes count was determined using microscope Neubauer counting chamber after diluting the blood with Hayems fluid at the ratio 0f 1: 200. Total numbers were reported as 10^6 mm^3 (Wintrobe, 1967).

2.14.4. Evaluation of white blood cell count

The leukocytes count was performed with improved Neubaur haemocytometer (Mgbenka *et al,* 2003) count chamber following dilution with Turkôs solution in the ratio of 1: 20. Four large corner squares were counted under the binoculars microscope and the total white blood cell calculuted in mm^3x10^3 (Wintrobe, 1967).

2.14.5. Evaluation of differential count (Wintrobe, 1967)

Differential leucocyte was estimated by dropping fresh blood on to one end of a clean grease-free slide, placed on a horizontal surface using a spreader, a little narrower than the slide. The drop of blood was spread along the slide until the blood was smeared. When the blood film was made, drying was hastened by waving in air. It was stained immediately using Leishman technique. This was done by adding 10 drops of Leishman stain on the dried smear and after two minutes, 20 drops of pH 6.8 distilled water was added. The stain with distilled water was allowed to dry and different cells were examined under oil immersion objectives of x100.

2.15. Determination of Blood Chemistry

2.15.1. Determination of sodium (Na^+)

Freshly collected blood from infected fishwas centrifuged at 12,000 rpm and the serum was collected. Into labeled test tubes A, B, C and D, 1.0 ml of filtrate reagent was added and then 50µl of sample was added to the test tube B,C and D while distilled water was added to the blank test tube (A). The tubes were shaken vigorously and mixed continuously for 3 minutes. They were centrifuged at 12,000 rpm for 10 minutes and the supernatant was tested ensuring that the protein precipitate was not disturbed.

Colour development: Into the labelled test tubes, 1.0ml of acid reagent followed by 50µL of supernatant and 50µL of color reagent were added and mixed. The absorbance of the labelled tubes was spectrophotometrically read at 550 nm and calculation was made thus:

Abs. of Blank-Abs. of Standard/Abs. of blank-Abs. of STD x Conc of STD (mEq/L) = Conc of Sodium (mEq/L).

2.15.2. Determination of chlorides (Cl^-)

Test tube A, B, C and D were labelled and 1.5ml chloride reagent was added into each labelled tube and 0.01ml (10µl) of calibrator or sample was added and mixed. The tubes were incubated at room temperature for 5 minutes. The absorbances of all the tubes were spectrophotometrically read at a wave length of 480 nm-520 nm**.** Calculation was made thus:

Abs. of unknown/ Abs. of calibrator x concentration of calibrator = concentration of chloride (mEq/L)

2.15.3. Determination of potassium (K^+)

Into labeled test tubes (A-D), 1.0 ml of potassium reagent as added. Then 0.01 ml (10µl) of sample was also added into the labeled tubes mixed and was allowed to stand for 3 minutes at 27°C room temperature . After 3 minutes, the absorbance was spectophotometrically read at the wavelength of 500 nm. Calculation was made thus; Abs. of unknown/Abs. of STD x Conc of STD (mEq/L) = Pottasium conc (mEq/L).

2.15.4. Determination of bi-carbonate (HCO_3)

To labeled test tubes A-D, 1.0 ml of carbon dioxide reagent was added and incubated for 3 minutes at the temperature of 37^{o}C. Pipette 5µl (0.005 ml) of water, standard and sample to the

cuvette labelled A, B and D mixed gently and incubated for 5 minutes. The absorbance was spectrophotometrically read at 340nm. Calculation was made thus:

CO_2 content of sample (mmol/L) = Abs. blank-Abs. sample/Abs. blank-Abs.standard x concentration of standard.

2.16. Biochemical Assay

2.16.1. Assay for catalase

Catalase was assayed according to the method of Takahara *et al* (1960). To 2ml of 0.01 mM phosphate buffer (pH 7.0), 0.5 ml of tissue homogenate (gill and muscle) obtained from the experimental fish were added. The enzyme reaction was started by the addition of 1.0 ml of 0.2 mM hydrogen peroxide solution. The decrease in absorbance was measured at 240nm for every 30 seconds up to 3 minutes. The enzyme blank was run simulteanoeusly with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as micro moles(µm) of hydrogen peroxide decomposed/minutes/ mg of protein.

2.16.2. Assay for superoxide dismutase

Superoxide dismutase was assayed according to the method of Misra and Fridovich (1972). A 0.1 ml of tissue homogenate (gill and muscle) was added to the tubes containing 0.75ml of ethanol and 0.15ml of chloroform under chilled condition and centrifuged t 12,000 rpm. To 0.5 ml of supernatant, 0.5 ml of 0.6 mM EDTA solution and 1.0 ml of 0.1 M carbonate-bicarbonate buffer (pH 10.2) were added. The reaction was initiated by the addition of 0.5ml of 1.8mM epinephrine and the increase in absorbance in 30 seconds interval for 3minutes was measured at 480 nm in UV spectrophotometer Jenway model 125. One unit of superoxide dismutase activity is the amount of protein required for 50% of inhibition of epinephrine autoxidation/minute.

2.16.3. Assay of gluthatione peroxidase

The activity of gluthathione peroxidase was assayed by the method of Rotruck *et al* (1973). The reaction mixture consisting of 0.2 ml of 0.2 ml of 0.8 mM EDTA, 0.1 ml of 10 mM sodium azide, 0.1 ml of 2.5 mM H_2O_2, 0.2 ml of GSH, 0.4ml of 0.4 mM phosphate buffer (pH 7.0) and 0.2ml of homogenate (gill and muscle) were incubated at 37^oC for 10 minutes. The reaction was arrested by the addition of 0.5 ml of 10% TCA and the tubes were centrifuged at 2000 rpm. To the supernatant, 3.0 ml of 0.3 M disodium hydrogen phosphate and 1.0 ml of dithio-bis-2-nitrobenzoic acid (DTNB) were added and the color developed was read at 420 nm immediately.

The activity of GP_x was expressed as micromoles (µm) of gluthathione oxidized/minute/mg of protein.

2.16.4.. Assay for Lipid Peroxidation as a Marker for Cellular Damage

Malondialdehyde contents were estimated according to the method of Buge and Aust (1978). To 1.0ml of the sample (gill and muscle homgenates), 2.0 ml of TCA-TBA_HCl reagent was added and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 12000 g for 10 minutes. The absorbance was determined at 535 nm against a blank that contained all the reagents except the sample. The results were expressed as nmoles of MDA formed/minute/mg protein using an extinction coefficient of the chromophore $1.56x10^5$ Mcm and expressed as nmoles of MDA formed/minute/mg protein.

2.16.5 Assay for myeloperoxidase enzyme

Total myeloperoxidase content present in the gill and muscle was measured according to Quade and Roth (1997). A 15 µl of gill and muscle were homogenized and centrifuged supernatant were diluted with 135µl of Hanks balanced salt solution (HBSS). Followed by 25 µl of 5mM of 3, 3ô-5, 5ô ï tetramethyl benzidine hydrochloride (TMB) and 25 µl of5mM H_2O_2. The colour change reaction was stopped after 2 min by adding 50 µl of 4M sulphuric acid. The Absorbance was read at 450nm spectrophotometrically**.**

2.16.6. Determination of serum total protein (Grant *et al.*, 1987)

Into labelled test tubes A - C, 0.02 ml of distilled water was added; test tube A (blank). A 0.02 ml of standard (CAL) was added into test tube B (Standard), 0.02 ml of the serum sample was added into the test tube labelled C while 1.0 ml of R1 (biuret reagent) was added into test tube A, B, C and incubated for 30 minutes at the temperature of 20-25^oC. The absorbance of the samples were measured at a wave length of 530 - 570nm and calculation was made thus;

Total Protein concentration (g/dl) = $A_{sample}/A_{standard}$x Standard concentration

2.16.7. Determination of serum albumin (Grant *et al.*, 1987)

Into labelled test tubes A-C, 0.01 ml of distilled water was added. Test tube A was blank, 0.01ml of standard (CAL) was added into test tube B (standard), 0.01 ml of serum sample was added into test tube labeled C while 3.0 ml of BCG reagent (R1) was added into tubes A, B and

C mixed and incubated for 5 minutes at the temperature of 20-25^{o}C. The absorbance was spectrophotometrically read at 630nm and calculation was made thus;

Albumin concentration (g/L) = $A_{sample} / A_{standard}$ x Concentration of standard

2.16.8. Determination of serum globulins

Total protein - Albumin = globulin (g/dl)

2.16.9. Determination of serum urea

Into labelled test tubes A - C, 10µl serum sample was added, followed by 10µl of standard (CAL) which was added into test tube B (standard) and 10µl of distilled water was added into test tube labeled C while 100 µl of reagent R1 (EDTA) was added into tubes A, B and C mixed and incubated for 10 minutes at the temperature of 37^{o}C. The absorbance was spectrophotometrically read at 546nm and calculation was made thus;

Urea concentration (mg/dl) = $A_{sample} / A_{standard}$ x Standard concentration

2.16.10. Determination of serum creatinine

Calibration graph for the estimation of cretainine was carried out in a 3ml reagent mixture containing 1.93 mM metol, 68.6µM copper and 1mM acetic acid/sodium acetate buffer of pH 5.4. The reaction was initiated by adding 100µL of creatinine concentrations. The reaction mixture was allowed to stand for 30 minutes at room temperature. Absorbance was read at 530nm (Copper and Bigga, 1961).

2.16.11. Determination of serum aspartate aminotransfarase (AST) (Reitman and Frankel, 1957)

The blood of the infected fish was collected through the caudal ablation and quickly transfered into blood container without EDTA. The serum was transferred into another test tube and immediately refrigerated so as to maintain the high level of enzyme activity until further analysis. 0.1 ml of the sample was pipetted into a test tube, mixed with 0.5 ml of buffer and incubated for exactly 1h at 37° C. 0.5 ml of the chromogen solution was mixed with the solution and allowed to stand for 20 min at 20° C to 25° C. After the time elapsed, 5.0 ml of 0.4N NaOH was added. The solution was allowed to stand for 5 minutes at room temperature; the absorbance was read against a blank using a colorimeter at a wavelength of 546 nm.

2.16.12. Determination of serum alanine transfarase (ALT) (Reitman and Frankel, 1957).

The blood of the fish was collected through the caudal ablation and quickly collectd with blood container without EDTA. The serum was transferred into another test tube and immediately refrigerated so as to maintain the high level of enzyme activity until further analysis.

0.1 ml of the sample was pipette into a test tube, mixed with 0.5 ml of buffer and incubated for exactly 30 min at 37° C. Also, 0.5ml of the chromogen solution was mixed with the solution and allowed to stand for 20 min at 20° C to 25° C. After the time elapsed, 5.0 ml of 0.4N NaOH was added. The solution was allowed to stand for 5 minutes at 27°C room temperature. The absorbances were read against a blank using colorimeter at wavelength of 546 nm.

2.16.13. Determination of alkaline phosphatase (ALP) (Randox Kit)

Into labelled tubes A-C, 0.05 ml of serum sample was added into test tube A, 0.02 ml serum sample was added into test tube B and 0.01 ml of serum sample into test tube C, while 3.0ml of reagent was added into test tube A, 1.0 ml in test tube B and 0.50 ml in test tube C. They were mixed thoroughly and incubated at 25^{o}C, 30^{o} C and 37^{o}C and initial absorbances read after 1, 2 and 3 minutes at 405 nm.

2.19.9. Determination of serum lactate dehydrogenase as a stress marker

Lactate dehydrogenase (LDH) was assayed according to the method of King (1965). To 0.1 ml of the buffered substrate, 0.1 ml of enzyme preparation was added and the tubes were incubated at 37^{o} C for 15 minutes. After adding 0.2 ml of NAD^{+} solution, the incubation was continued for another 15 minutes. The reaction was arrested by addition of 1.0 ml of DNPH reagent and then the tubes were incubated for further period of 15 minutes at 37^{o} C. After the incubation period, 7.0 ml of 0.4 N sodium hydroxide solutions was added and the color developed was measured at 420 nm in a spectrophotometer. Suitable aliquots of the standards were also analyzed by the same procedure. The activity of the enzyme was expressed as micromoles of pyruvate formed/hour/mg of protein for tissues under incubation conditions.

2.17. Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 16 was used. Mean values was analyzed for significant differences ($P < 0.05$) using the analysis of variance (ANOVA). Differences between means were partitioned using the Duncan New Multiple Range test.

CHAPTER THREE

RESULTS

3.1. Estimation/Counting of Parasites of Infested Fish

The estimation of *I. multfiliis* parasites observed from Infected fish treated with aqueous extracts of *M. oleifera* leaves showed significant changes. The appearance of white spots, trophonts (adult parasites) both in the gills and body smear of infected fish that were enumerated to ascertain any possible changes in their number before and after treatment are presented in Figures 2 and 3.

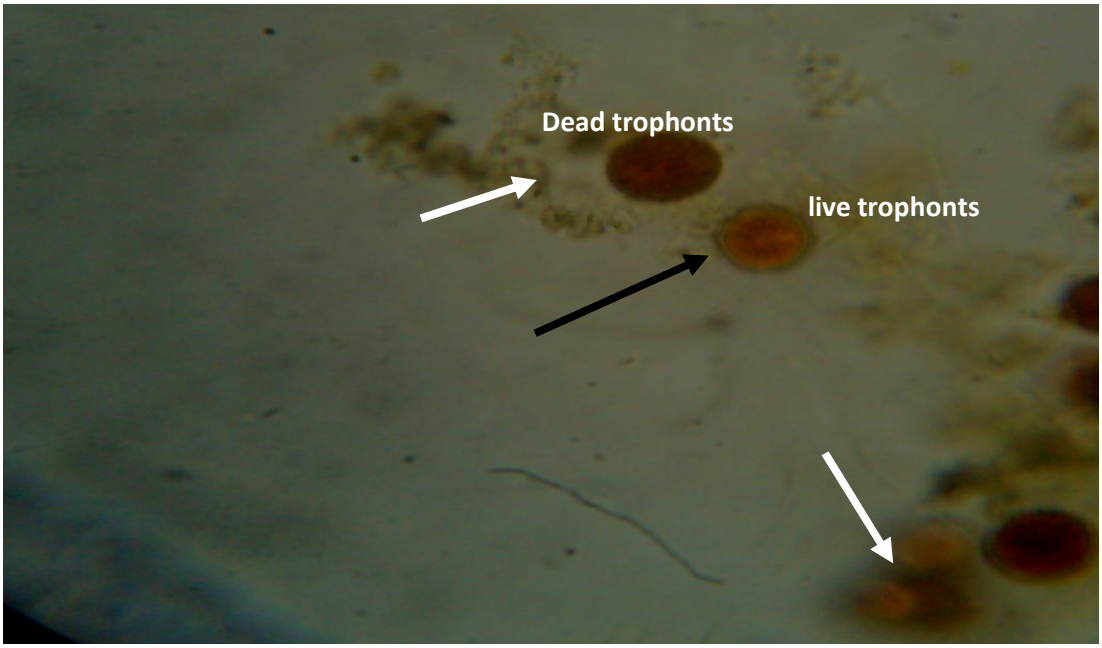

Figure 2. Gill of fish showing dead (white arrow) and live (black arrow) trophonts with intact nucleus under lugol iodine stain during treatment with aqueous extracts of *Moringa oleifera*. Mag. 100X

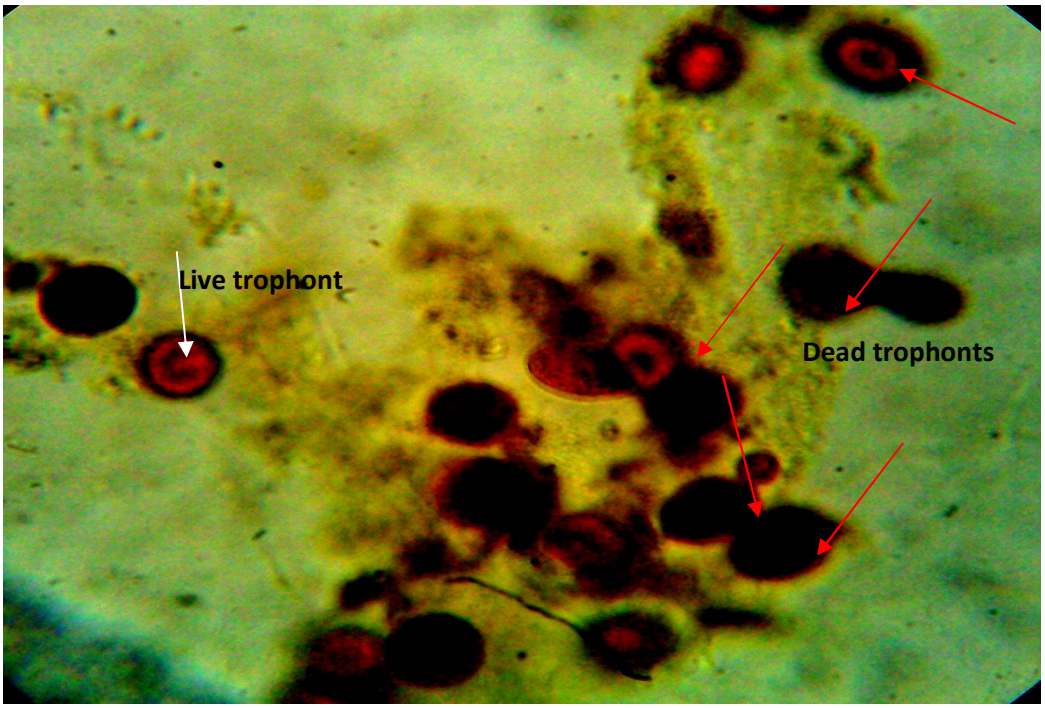

Figure 3. Body smear of fish showing dead (red arrow) and live trophonts (white arrow) with intact nucleus under lugol iodine stain during treatment with aqueous extracts of *Moringa oleifera*. Mag. 100X.

3.1.1. White spots and trophonts number present in the body, body smear and gills of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* before and after dip treatment (1h) with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure)

The results showed that the dip treatment with the *M. oleifera a*queous leaves extracts showed non-significant ($P>0.05$) concentration dependent increase in the number of white spots compared to the healthy control (group A). There was no significant difference ($P>0.05$) in the number of white spots between the treated groups and the negative control. However, during the dip bath post-treatment, the white spots were significantly reduced ($P<0.05$) in the positive control. However, other treated groups (D - F) showed a decreasing trend of white spots in comparison with infested not treated group (Table 2). The decrease appeared to be concentration and time dependent.

Table 2: White spots present in the body of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* before and after dip treatments (1h) with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before treatment	After treatment
A (Healthy control)		-
B (infested not treated)	519.00 ± 186.24^{a1}	361.33 ± 86.35^{bc2}
C (standard drug)	399.00 ± 175.69^{a1}	241.33 ± 130.16^{b2}
D (1,500mg/l)	678.33 ± 185.511^{a1}	498.67 ± 67.87^{c2}
E (2,500mg/L)	513.667 ± 147.24^{a1}	286.67 ± 99.68^{b2}
F (3,500mg/L)	482.667 ± 142.24^{a1}	275.00 ± 126.74^{b2}
G (4,500mg/L)	601.0 ± 106.86^{a1}	268.28 ± 164.17^{b2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

Results from the number of trophonts present in the gill during pre treatment showed no significant difference ($P>0.05$) in the treated groups compared to negative control that had elevated trophonts in the gill. Moreover, the healthy control group did not show any presence of trophonts in the gill. During the dip bath-post treatment, it was observed that the number of trophonts present in the gills of the infested fish was statistically significant ($P<0.05$) especially in untreated group. Meanwhile, the group treated with standard drug and 4,500 mg/L *M. oleifera* aqueous extracts showed decreased number of adult trophonts in comparison with group B which had an increased number of trophonts present in the gills. Moreso, the group D, E and F had a mixed trend of increased adult trophonts as shown in Table 3.

Table 3: Trophonts present in gill of *Clarias gariepinus* infested with *Ichthyophthirius multifiliis* before and after dip treatments (1h) with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Trophonts present in gills	
	Before treatment	After treatment
A (Healthy control)	-	-
B (infested not treated)	9.10 ± 4.58^{ab1}	20.33 ± 10.50^{b2}
C (standard drug)	4.33 ± 5.86^{ab1}	2.00 ± 2.00^{a2}
D (1,500mg/L)	9.33 ± 8.32^{ab1}	5.33 ± 3.51^{a2}
E (2,500mg/L)	12.67 ± 1.53^{b1}	8.10 ± 2.65^{a2}
F (3,500mg/L)	12.67 ± 1.53^{b1}	4.67 ± 5.03^{a2}
G (4,500mg/L)	12.00 ± 4.58^{b1}	2.10 ± 2.00^{a2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

The trophont burdens on the experimental fish were determined at one time points, 1h after initial infestation, when only the triplicate set of tanks (C - G) received treatment. Results showed that decrease in the number of trophonts present in the body smear was concentration and time dependent (Table 4). It was observed that trophonts present in body smear during pre-treatment were significantly ($P<0.05$) higher among the experimental groups, especially the infested not treated which had the highest number of trophonts in the body smear of the infested fish. In the treated groups, the number of observed trophonts in the body smear was 47.67±5.13, 82.33±44.16, 55.00±12.72, 25.33±13.61, 40.67±14.14, for group C (standard drug), group D (1,500mg/L), group E (2,500mg/L), group F (3,500mg/L) and group G (4,500mg/L), respectively. The lowest number of trophonts in the body smear of the infested fish was recorded in group F (Table 4). However, at the end of the post-treatment, the number of trophonts in the infested not treated increased significantly ($P<0.05$) when compared to other treated groups. The trophont populations in the body smear of the infested fish differed significantly ($P<0.05$) among all the treatments, although, group D and F had similar increase in their trophont numbers (Table 4). However, the group C treated with standard drug had a significantly decreased trophonts with mean value 1.67±0.57, followed by group G, 14.33±2.52 and group E 17.00±0.65 at the end of the dip treatment that lasted for 1h. Morever, the trophont levels decresed in a concentration and time dependent manner among the extracts treated groups and standard drug at the end of the dip treatment when compared to the negative control that had increased number of the adult parasite (trophont).

Table 4: Trophonts in the body smear of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* before and after dip treatments (1h) with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Mean number of trophonts before treatment	Mean Number of trophonts after treatment
A (Healthy control)	-	-
B (Infested not treated)	84.33 ± 43.9^{c1}	258.00 ± 44.53^{b2}
C (Standard drug)	47.67 ± 5.13^{bc1}	1.67 ± 0.57^{a2}
D(1,500mg/L)	82.33 ± 44.16^{c1}	24.67 ± 21.13^{a2}
E (2,500mg/L)	55.00 ± 12.77^{bc1}	17.00 ± 2.65^{a2}
F (3,500mg/L)	45.33 ± 13.61^{ab1}	27.00 ± 11.14^{a2}
G (4,500mg/L)	40.67 ± 14.15^{abc1}	14.33 ± 2.52^{a2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.1.2. Curative potential of aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) in eliminating the adult parasite trophonts from the body smear of the infested fish, after dip treatments

Results showed that curative potency of aqueous extracts of *M. oleifera* in the number of trophonts present in the body and gill of the infested animal were significantly different at ($P<0.05$). The depletion of trophonts in the curative potentials of the extracts was conentration dependent. In the positive control, the percentage curative of trophonts in the body was 99.32±0.33%, followed by group G with mean value 94.17±2.14% and group E 93.16±2.19%, group D, 92.02±9.26% and group F, 89.77±2.63%. Moreover, there was no positive curative change in group B (infested not treated) when compared to other treated groups (Table 5).

Similar findings were also observed in the gills of the infested fish where the positive control group and group G (4,500mg/L) had similar percentage curative potentials when compared to other groups. The mean percentage curative in the gills of the infested fish in group C and G were 83.33±20.82% and 83.33±20.82%, respectively. The lowest *M. oleifera* concentration 1,500mg/L and 2,500mg/L did not show any significant curative potentials in the trophonts numbers in the gill of the infested fish, but at the highest concentrations (3,500mg/L and 4,500mg/L), the trophonts numbers in the gills were significantly ($P<0.05$) eradicated by 79.03±25.96% to 83.33±20.82%, respectively when compared to the negative control (infested not treated). The percentage curatives of *M. oleifera* aqueous extracts on the various treated groups were concentration and time dependent.

Table 5: Changes in the curative potential of *Moringa oleifera* aqueous extracts and standard drug (fish cure) in eliminating the adult parasite trophonts from the body smear of the infested fish, after dip treatments

Experimental group	Percentage (%) of curative trophonts present in the gill of infested fish	Percentage (%) of curative trophonts alive and present in the body of the infested fish.
Group B(infested not treated)	0.00 ± 0.00^{a2}	0.00 ± 0.00^{a1}
Group C(standard drug)	83.33 ± 20.82^{b1}	99.32 ± 0.33^{c2}
Group D(1,500mg/L) extracts	66.17 ± 23.80^{b1}	92.02 ± 9.26^{b2}
Group E (2,500mg/L) extracts	54.83 ± 21.68^{b1}	93.16 ± 2.19^{bc2}
Group F (3,500mg/L)extracts	79.03 ± 25.96^{b1}	89.77 ± 2.63^{b1}
Group G (4,500mg/L) extracts	83.33 ± 20.82^{b1}	94.17 ± 2.14^{bc2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.2. Changes in the white Spots and Trophonts Number Present in the Body, Body Smear and Gills of *Clarias gariepinus* Infested with Ich Before and After Short Term Treatments (1h) with Aqueous Extracts of *Moringa oleifera* Leaves and Standard Drug (fish cure).

Results showed that from the baselines, the number of white spots observed during the pre-treatment showed no significant difference ($P>0.05$). The infested fish treated with graded concentrations of *M. oleifera* in a short-term bath treatment showed that at the end of 24h of exposure, the number white spots in group D, E, F and G were 777.33±352.99, 516.33±259.11, 619.67±312.96 and 421.33±63.0, respectively. The number of white spots in group B at the end of 24 h exposure was observed to be significantly higher ($P<0.05$) in comparison to other treatment groups. At the same time at the end of 24h, the number of observed white spots in the treatment groups and negative control were significantly different ($P<0.05$). Moreover, at the end of 48h, among the treatment groups, it was observed that the number of white spots differed significantly ($P<0.05$) within the groups (Table 6).

Table 6: Mean number of white spots ±SD per fish present in the body of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* during short term treatment (24 – 96 h) with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Number of white spots before treatment	Number of white spots after 24 h treatment	Number of white spots after 48h	Number of white spots after 72h	Number of white spots after 96h
Group A(Healthy control)	-	-	-	-	-
B (Infested not treated)	538.67 ± 208.53^{b1}	1161.10 ± 271.22^{c2}	$874.67\pm4.14^{c3,1}$	537 ± 67178.31^{de4}	411.33 ± 963.83^{b5}
C (Standard drug)	375.33 ± 111.87^{b1}	732.10 ± 360.34^{bc2}	499.67 ± 277.27^{bc2}	231.0 ± 77.09^{b4}	207.33 ± 31.34^{ab5}
D (150 mg/L)	346.33 ± 108.19^{b1}	777.33 ± 352.99^{bc2}	371.67 ± 73.79^{ab1}	387.33 ± 68.63^{bc1}	373.67 ± 96.09^{a4}
E (250 mg/L)	385.0 ± 75.35^{b1}	516.33 ± 259.11^{b2}	469.00 ± 121.89^{b2}	461.33 ± 130.54^{cde2}	291.67 ± 87.69^{a1}
F (350mg/L)	348.67 ± 106.80^{b1}	619.67 ± 312.96^{b2}	502.67 ± 204.59^{bc2}	604.67 ± 136.05^{e2}	$277.67\pm182.43a^{1}$
G (450mg/L)	437.33 ± 230.10^{b1}	421.33 ± 63.0^{ab1}	275.33 ± 50.14^{ab2}	270.67 ± 57.20^{bc2}	244.67 ± 85.58^{ab1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

The trophonts burdens on the experimental fish were determined at 4 time points 24h after initial infestation and then 48h, 72h and 96h post infestation to determine the relative increase or decrease during the treatment period.

Complete eradication of the adult parasites was recorded at the end of 96h only in the positive control when compared to the varied concentration of extractss. As shown in Table 7, it was observed that the high level of trophonts present in the body smear of the infested fish declined throughout the duration of exposure and among the experimental groups. From the baseline (before treatment), it was observed that the number of trophonts present in the body smear of the infested fish showed no significant difference ($P>0.05$) when compared to the normal control. During the short term treatment, various experimental groups showed changes in their mean trophont numbers. For example, along the duration of exposure, the group treated with standard drug showed a decline in trophont number from 4.10±4.36 trophonts to 0.00±0.00 showing complete eradication of the trophonts on the body of the fish although at the end of 72h, it appeared that the trophont number present in the examined infested fish increased when compared to the before treatment. Similarly, the same trends were observed among other experiential groups. The group D treated with 150mg/L extracts showed that the trophont number significantly decreased from 46.00±6.24 to 17.00±0.00; in group E treated with 250 mg/L extractss, trophonts number decreased from 48.33±16.01 to 16.67±2.52, group F (350 mg/L) trophonts declined from 63.33±7.50 to 17.33±5.03 while group G treated with 450 mg/L of the aqueous extracts showed similar progressive reductions of the adult parasite, from 56.67±14.29 to 14.10±3.00 when compared to the negative control that maintained a progressive increase in the number of the adult parasites throughout the study period. The level of

eradication of the adult trophonts, present in the infested fish varied among the experimental groups. However, in 24h exposure the trophonts reduced drastically in the group treated with standard drugs, with mean value 4.00±4.36, followed by the group G treated with the highest concentration of the extracts (450 mg/L) with mean value, 7.67±5.51, followed by group F treated with 50mg/L with mean value 10.33±3.5, followed by group E treated with 250mg/L with mean value 14.00±7.00, and while the highest number of trophonts was recorded in group D treated with 150mg/L with mean value 21.00±12.12. The trophonts in the body smear were statistically significant at $P<0.05$ at the end of 24h exposure. Moreover, at the end of 48h, among the experimental groups, they showed a significant decrease ($P<0.05$) in the trophonts number especially with the positive control (treated with standard drug) < group F (350 mg/L) <group G (450 mg/L) <group E (250 mg/L) <group D (150 mg/L) the infested not treated. At the end of 72h, there was a significant decrease in the trophont numbers present in the body smear within the experimental groups in comparison to the negative control. At all concentrations, *M. oleifera* was able to reduce, the number of trophonts present in the body of the infested fish, through the reductions varied in all the concentrations together with the standard drug Table 7.

Table 7: Parasiticidal activity of aqueous extracts of *Moringa oleifera leaves* and standard drug (fish cure) in eliminating trophonts present in body smear of *Clarias gariepinus* Infested with *Ichthyophthirius multifilis* during short-term treatments.

Experimental group	Mean number of trophonts before treatment	Mean number of trophont present 24h	Mean number of trophont present 48h	Mean number of trophonts present 72h	Mean number of trophonts present 96h
A	-	-	-	-	-
B	57.67 ± 17.95^{b1}	60.62 ± 1180^{c2}	131.33 ± 56.36^{c3}	$124.67\pm41.47^{c4,2}$	155.67 ± 55.34^{b5}
C	46.10 ± 13.07^{b1}	4.00 ± 4.36^{a2}	8.0 ± 2.61^{a3}	10.67 ± 3.21^{a4}	0.10 ± 0.00^{a5}
D	46.10 ± 6.24^{b1}	21.10 ± 12.12^{b2}	39.33 ± 8.6^{a3}	58.00 ± 41.79^{b4}	17.00 ± 3.00^{a5}
E	48.33 ± 16.01^{b1}	14.00 ± 7.00^{ab2}	29.33 ± 8.74^{a3}	$31.33\pm15.14^{ab4,3}$	$16.67\pm2.52^{a5,2}$
F	63.33 ± 7.50^{b1}	10.33 ± 3.51^{ab2}	24.00 ± 8.72^{a3}	27.67 ± 9.87^{ab3}	17.33 ± 2.52^{a4}
G	56.67 ± 14.29^{b1}	7.67 ± 5.51^{ab2}	24.67 ± 11.15^{a3}	19.00 ± 2.00^{ab4}	$14.00\pm3.00^{a5,4}$

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$). A (normal control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

The effect of aqueous extracts *M. oleifera* leaves and the degree of changes of parasite infestation in *C. gariepinus* was compared and recorded in Table 8. The results of the effect of the ecto protozoal infestation in the gills of the infested fish, showed that at the baseline (before treatments), the trophonts present in the gills of all the fish were not significantly different ($P>0.05$) which depicted uniform distribution of the adult parasites. Moreover, the group C (standard drug) showed a quick eradication or knock off potential of the trophonts completely from the gill. The group C had a decline of trophont numbers from 2.33±4.04 at 24h to 0.00±0.00 at 72 and 96h, and thus showed effective potency of the standard drug. At the same time, throughout the duration of exposure the varied concentrations of extractss (D - G) showed a reduction in the trophont numbers for example; the trophonts in the infested fish in group D reduced from 8.33±6.03 at 24h to 6.00±0.00 at 96 h, group E reduced from 9.33±4.16 at 24h to 0.00±0.00 at 96 h, group F, reduced from 4.67±3.06 at 24h to 0.00±0.00 at 96h. The effectiveness of the extracts treated groups cannot be compared to the standard drug because at the end of 72h, there was a zero recorded number of trophonts in group C compared to the extracts groups which were not able to eradicate the trophonts at 72h but did so at 96h. Along the column, at the end of 24h, there was a significant ($P>0.05$) reduction in the number of trophonts present in the gills of the infested fish with positive control (group C) having the least number of trophonts with mean value of 2.33±4.04 to group F (350 mg/L) treated group with highest mean value of trophonts to be (13.33±2.53). Similarly, at the end of 48h, the positive control group had the least number of trophonts, followed by group E, group G, group F and group D and the decrease was not dose dependent. There was a significant difference ($P<0.05$) at the end of 48h exposure among the experiment groups when compared to the negative control at 72h exposure. The standard drug was able to eradicate the trophonts present in the gill in comparison with other

extracts treated groups that showed a decline of trophonts population by 2.33±1.53, 2.33±0.00, 2.67±0.58 and 3.67±3.06 as recorded in groups G, E, F and D, respectively. Moreso, the negative control maintained a progressive increase in trophont present in the gill of the infested fish. At the end of the 96h exposure, there was complete eradication of the adult parasites initially found in the gills of the infested fish before treatment. The short term bath treatment with the plant extracts showed that ectoprotozoal parasite could be significantly wiped off from the body of the infested fish within a short period and at varied concentrations Table 8.

Table 8: Parasiticidal activity of aqueous extracts of *Moringa oleifera* leaves on the trophonts present in gill of *Clarias gariepinus* infested with *Ichthyophthirius multifilis during* short-term treatment (24 - 96h) with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental goup	Number of trophonts before treatment	Number of trophonts after 24h	Number of trophonts after 48h	Number of trophonts after 72h	Number of trophonts after 96h
A	-	-	-	-	-
B	00.00 ± 3.00^{b1}	18.00 ± 3.61^{c2}	11.33 ± 2.08^{c3}	18.00 ± 7.21^{b2}	$21.00\pm2.65^{b3,2}$
C	11.33 ± 8.74^{b1}	2.33 ± 4.04^{ab2}	1.00 ± 1.00^{ab2}	0.0 ± 0.00^{a3}	0.00 ± 0.00^{a3}
D	8.67 ± 0.58^{b1}	8.33 ± 6.03^{bc1}	4.10 ± 2.65^{b2}	3.67 ± 3.06^{a2}	0.00 ± 0.00^{a3}
E	10.10 ± 5.19^{b1}	9.33 ± 4.16^{bc1}	2.67 ± 1.15^{ab2}	2.33 ± 0.58^{a2}	0.00 ± 0.00^{a3}
F	8.00 ± 3.46^{b1}	13.33 ± 2.52^{cd2}	3.33 ± 2.31^{ab3}	2.67 ± 0.58^{a3}	0.00 ± 0.00^{a4}
G	7.33 ± 1.53^{ab1}	4.67 ± 3.06^{ab2}	3.00 ± 1.73^{ab2}	$2.33\pm1.53^{a3,2}$	0.00 ± 0.00^{a3}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.2.1. Percentage cure in the gills and body smear of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* during short term treatments in relation to the untreated fish with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Results showed that the administration of varied concentrations of aqueous extracts of *M. oleifera* in the aquaria containing infested fish caused a markedly significant ($P<0.05$) eradication of the protozoal parasite (Ich) from the fish. But Ich parasites were still present in the negative control group. During the study it was observed that parasites present in the gills were completely eradicated by the standard drug with a significant progressive curative percentage from 87.716±21.27% to 100.00±0.00%; followed by the group G treated with highest concentration of the extracts which had a curative percentage from 74.85±74.87% to 89.00±6.63%, whereas, group D, and F had a similar curative percentages was from 47.70±45.44% to 88.41±2.87%, 43.103±37.94% to 88.07±5.38% and 34.04±23.29 % to 88.49±2.21%.

Along the column, it was observed that percentage cure of the trophonts present in the gills at the end of 24h within the experimental groups differed significantly at $P<0.05$ showing that the use of herb (*M. oleifera*) had positive effect on the eradication of the protozoal parasite in the gill of infested fish. Moreso, during 48h exposure period, significant differences ($P<0.05$) were recorded in the percentage cure of adult parasites. The same was observed at 72h and 96h exposures and significant differences ($P<0.05$) were observed among the experimental groups in eradicating the parasite in the gills of the infested fish as shown in Table 9.

In the body smear of the fish, the trophonts numbers were significantly eradicated at the end of 96h exposure with 100% curative efficiency among all the experimental groups, in comparison

with the negative control. Throughout the duration of exposure (24-96h), significant difference ($P>0.05$) were recorded among experimental groups (Table 10).

Table 9: Percentage cure in the gills of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* during short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental groups	% cure 24h	% cure 48h	% cure 72h	% cure 96h
Group A (healthy control)	-	-	-	-
B (infested not treated)	0.0 ± 0.0^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.10^{a1}	0.00 ± 0.0^{a1}
C (standard drug)	87.716 ± 21.27^{e1}	91.163 ± 7.94^{c1}	100.10 ± 0.10^{c2}	$100.10\pm0.00ac^{c2}$
D (150mg/L)	47.70 ± 45.44^{abc1}	65.45 ± 20.59^{b2}	70.27 ± 35.29^{b3}	88.41 ± 2.87^{b4}
E (250mg/L)	43.103 ± 37.94^{abc1}	78.62 ± 10.35^{bc2}	85.53 ± 5.82^{bc3}	88.07 ± 5.38^{b3}
F (350mg/L)	34.04 ± 23.29^{ab1}	71.65 ± 15.67^{bc2}	78.87 ± 7.36^{bc2}	88.49 ± 2.21^{b3}
G (450mg/L)	74.85 ± 14.87^{bc1}	74.93 ± 12.16^{bc1}	85.27 ± 9.14^{bc2}	89.40 ± 6.63^{b3}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

Table 10: Percentage cure in the body smear of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* during short term treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	% cure 24h	% cure 48h	% cure 72h	% cure 96h
Group A (healthy control)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00
B (infested not treated)	0.00 ± 0.00^{a1}	$0.00\pm.00^{a1}$	0.00 ± 00^{a1}	0.00 ± 0.00^{ab1}
C (standard drug)	93.46 ± 7.13^{c1}	93.95 ± 1.13^{c1}	91.23 ± 2.47^{c1}	100.10 ± 0.10^{ca2}
D (150mg/L)	64.49 ± 17.54^{b1}	64.95 ± 21.21^{b1}	56.77 ± 27.52^{b2}	100.10 ± 0.10^{a2}
E (250mg/L)	75.73 ± 12.86^{bc1}	75.88 ± 8.82^{b1}	70.85 ± 18.27^{bc1}	100.00 ± 0.0^{a2}
F (350mg/L)	84.51 ± 4.47^{c1}	81.3 ± 3.49^{bc1}	74.90 ± 15.69^{bc1}	100.00 ± 0.0^{a2}
G (450mg/L)	87.8 ± 8.71^{c1}	81.37 ± 4.26^{bc1}	83.33 ± 661^{b1}	100.00 ± 0.0^{a2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.2.2. Knockout efficacy of aqueous extracts *Moringa oleifera* leaves and standard drug (fish cure) after 96h of exposure to *Clarias gariepinus* infested with *Ichthyophthirius multifilis*

From the foregoing, results showed that during the treatment periods, the presence of dead trophonts/30mls of the water were observed. However, the number of dead trophonts which was assumed to be present in the water at the end of the 24h showed a significant difference ($P<0.05$) in the knockout of parasites. The group C treated with standard drug showed the highest knockout potential of the drug to the parasite with a mean number of 73.50±6.29 but when compared with the group G with highest concentration of the extracts, there was a slight variation in their knockout percentages when compared to other treated groups. Moreover, group D treated with 150mg/L of the extracts, group E treated with 200mg/L and group F treated with 350mg/L showed varied reduced knockout potentials when compared to the standard drug and the group G (450mg/L) extracts treated group.

Moreover, at the end of 96h, all the trophonts seen in the water were dead and their knockout percentages were significantly different at $P<0.05$. Hence, there is 100% percent or complete knockout of the adult parasites from the body and gill of the infested fish due to the standard drug and the varied concentrations of the extracts. The need for the percentage knockout in water per 30mls was because of the need to truncate any possible bias that may emanate due to the enumeration of the trophonts in the gills and body smear only (Table 11).

Table 11: Percentage knockout efficacy of aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) after 96h of exposure of *Clarias gariepinus* to *Icthyophthirius multifilis*

Experimental group	% knockout at the 24h	% knockout at the end of 96h
Group A (healthy control)	-	-
B (infested not treated)	0.00 ± 0.00^{b1}	0.00 ± 0.00^{b1}
C (standard drug)	73.50 ± 6.29^{c1}	100.00 ± 0.00^{a2}
D (150mg/L)	16.67 ± 28.87^{a1}	100.00 ± 0.00^{a2}
E (250mg/L)	14.28 ± 24.75^{a1}	100.00 ± 0.00^{a2}
F (350mg/L)	40.75 ± 65.08^{ac1}	100.00 ± 0.00^{a2}
G (450mg/L)	80.79 ± 1242^{c1}	100.00 ± 0.00^{a2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.3. Parasiticidal Activity of Aqueous Extracts of *Moringa oleifera* Leaves and Standard Drug (fish cure) on White Spots Present in the Body Smear and Gills of *Clarias gariepnus* Infested with *Ichthyophthirius multifilis* before and after Prolonged Bath Treatments.

The results of the pre-treatment study on C. *gariepinus* infested with Ich showed a significant increase in the number of protozoan parasites when compared to the group A (Healthy control) (Table 12). The number of white spots observed before treatment in group B -G were not significant ($p>0.05$), but differed significantly ($P<0.05$) when compared with the normal control group. During the prolonged bath treatments, there was a mixed trend in the group B (infested not treated) with mean values of 690.33±972.21, 96.94±55.97 and 615.67±88.67 pre treatments on day 5 and day 15, respectively. Similar changes was observed in the extracts treated group E with mean white spots values of 601.33±93.15, 531.67±61.33 and 648.00±224.33, for post-treatments on day 7 and day 15. At the same time, group F had the same pattern of change with 606.00±187.10, 7868.80±177.28 and 588.67±170.17 white spots (Table 12). The group treated with 450mg/L of the extracts showed the same trend with values of 634.33±170.12, 639.67±71.44 and 527.10±292.96 for pre treatments on day 7 and day 15 exposure, respectively. In contrast, groups C and D showed progressive decline in the number of white spots with values of 1004.00±183.86, 609.00±75.62 and 562.00±44.65 and 611.50±17.68 in group D for post-treatments on day 7 and day 15, respectively.

The white spots in infested fish before treatment were significantly different ($P<0.05$) within the experimental groups. More so, in day 7 and day 15 the white spots values differed significantly at $P>0.05$ among the experimental groups Table 12.

Table 12: Parasiticidal activity of aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) on white spots present on the body of *Clarias gariepinus* infested with *ichthyophthirius multifilis* before and after prolonged bath treatments.

Experimental group	Pre treatment	Day 7	Day 15
Group A (healthy control)	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}
B (infested not treated)	690.33 ± 172.21^{b1}	96.94 ± 55.97^{bc2}	615.67 ± 88.67^{b3}
C (standard drug)	1004.10 ± 183.86^{a1}	609.00 ± 75.62^{bc2}	562.50 ± 17.68^{b3}
D (150mg/L)	834.67 ± 466.10^{b1}	753.33±44.65c2	611.50 ± 17.68^{b3}
E (250mg/L)	601.33 ± 93.15^{b1}	531.67 ± 61.33^{b2}	649.00 ± 224.83^{b3}
F (350mg/L)	606.00 ± 187.10^{b1}	768.50 ± 197.28^{c2}	588.67 ± 170.17^{b3}
G (450mg/L)	$634.33+170.12^{b1}$	639.67 ± 71.44^{bc1}	527.10 ± 292.96^{b13}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.3.1. Parasiticidal activities of aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) in trophonts present in gill of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* during prolonged bath treatments (5 - 15 days)

The results presented in Table 13 showed the parasiticidal activity of *M. oleifera* on trophonts present in the gill of the ich infested fish. The normal control showed absence of the parasite in the gill of the fish. Following the treatment with aqaeous extracts of *M. oleifera*, changes in the number of the trophonts were noted particularly in the gill (Table 13). The infested fish in group D treated with 150mg/L extracts showed a decline in trophonts present in the gill from 5.67±3.05 to 3.00±1.00. The positive control group also had reduced number of trophonts in the gills of infested fish from 4.00±3.00 across the duration. The infested fish in groups E, F and G reduced from 2.67±1.15 to 3.10 ±1.00, 8.00±3.61 to 2.33±1.15 and 9.00±8.19 to 3.00±1.00, respectively. The positive control group also had reduced number of trophonts in the gills of infested fish from 4.00±3.00 to 1.00±1.00. In day 7 and day 15, there were significant changes $P<0.05$ in the number of trophonts present in the gills of infested fish in the treated groups. Hence, the no significant difference ($P>0.05$) observed in the pre-treatments which confirmed the uniformity of the parasites penetrations in the exposed fish. However, during the end of the treatment period especially on day 15, significantly low ($P<0.05$) trophonts were recorded in the different treatment groups in comparison with the negative control.

Table 13: Parasiticidal activities of aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) on trophonts present on gill of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* during prolonged bath treatments (5 - 15 days)

Experimental group	Pre treatment	Day 7	Day 15
Group A (healthy control)	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}
B (infested not treated)	7.33 ± 4.73^{ab1}	17.33 ± 3.51^{c2}	$10.67\pm208^{d3,1}$
C (standard drug)	4.10 ± 2.00^{ab1}	2.67 ± 2.31^{ab1}	1.00 ± 0.00^{ab1}
D (150mg/L)	5.67 ± 3.05^{ab1}	5.00 ± 2.65^{ab1}	4.10 ± 1.00^{ab1}
E (250mg/L)	2.67 ± 1.15^{ab1}	5.67 ± 2.08^{b1}	3.00 ± 1.00^{bc1}
F (350mg/L)	8.00 ± 3.61^{ab1}	6.67 ± 4.51^{b1}	2.33 ± 1.15^{abc2}
G (450mg/L)	9.00 ± 819^{b1}	6.10 ± 1.73^{b1}	3.00 ± 1.00^{bc1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.3.2. Parasiticidal activities of aqueous extracts of *M. oleifera* leaves in trophonts present in body smear of *C. gariepinus* infested with ich during prolonged bath treatments (5 - 15 days)

Table 14 shows changes in the number of trophonts present in the body smear of the infested fish exposed to graded concentrations of aqaeous extracts of *M. oleifera* and standard drug for 15 days. It was observed that before treatment, the number of trophonts in the infested fish randomly distributed into various experimental groups were significantly difference ($P>0.05$). During the prolonged bath treatment, it was observed that throughout the exposure period especially in day 7 and day 15, there were a significant differences ($P<0.05$) in the number of trophonts present in the body smear of infested fish among the experimental groups. The infested untreated groups did not show much changes in the trophonts present in the body smear of the infested fish but only differed significantly ($P<0.05$) in day 15. The reduction in the trophonts depended on the duration of exposure and concentration of the extracts used. This coincided with the markedly significant ($P>0.05$) reduction of trophonts in the body smear of infested fish observed in group C, group D, group E, group F and group G with varied values of 69.67±11.59 trophonts to 0.00±0.00, 44.33±13.05 to 1.67±1.53, 77.67±16.33 to 2.00±1.00, 98.00±18.33 to 1.33±2.31 and 50.00±11.53 to 1.33±0.58 trophonts, respectively; whereas no trophonts was found in the body smear of group A (normal control). In addition, the group C treated group had 100% eradication of the trophonts from the body smear due to zero mean value recorded in day 15. The range of *M. oleifera* aqueous extracts concentrations on the trophonts present in the gills, demonstrated a distinct concentration-response and time relationship.

Table 14: Trophonts present in the body smear of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* during prolonged bath treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Pre treatment	Day 7	Day 15
A(Healthy control)	0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}
B (infested not treated)	81.67 ± 13.65^{de1}	34.33 ± 9.0^{ac2}	9.67 ± 3.0^{b3}
C(standard drug)	69.67 ± 11.59^{cd1}	4.00 ± 2.00^{ab2}	0.00 ± 0.00^{a3}
D(15mg/L)	44.33 ± 13.05^{b1}	9.00 ± 2.00^{b2}	1.67 ± 1.53^{a3}
E (25mg/L)	77.67 ± 16.33^{de1}	10.67 ± 3.08^{b2}	2.00 ± 1.00^{a3}
F 35mg/L)	98.00 ± 18.33^{f1}	8.00 ± 4.58^{b2}	1.33 ± 2.31^{a3}
G (45mg/L)	50.10 ± 11.53^{bc1}	9.33 ± 2.52^{b2}	1.33 ± 0.58^{a3}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.3.3. Percentage cure of aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) on *Clarias gariepinus* infested with *Ichthyophthirius multifilis* parasites during prolonged bath treatments.

During the prolonged bath treatments, the varied concentration of *M. oleifera* extracts used in this study showed that group F treated with 35 mg/L of the extracts had the highest percentage range cure of 77.67±8.25% to 89.73±17.78% in eradicating the trophonts in the body smear of the infested fish while group E, group D and group G had the lowest percentage range cure of 66.47±20.72% to 80.11±4.88, 71.22±14.92% to 82.77±15.18% and 93±205% to 86.37±2.17% respectively between day 7 and day 15. When compared with the standard drug, the group C had the highest eradicated trophonts with percentage mean value range of 88.78±3.16% to 100.00±0.00% in day 7 and day 15, respectively.

There was a significant difference ($P<0.05$) between the treated groups in eradication of torphonts present in the body smear of the infested fish in day 7 and day 15. Along the column in day 7, the standard drug had the highest percentage cure of 88.78±3.16%, followed by group E with percentage curative mean value of 77.67±8.25%, followed by group G with percentage curative mean value of 71.93±2.05%, while group D and group E had percentage curative mean values of 71.22±14.92% and 66.47±20.72%, respectively. At the end of the prolonged bath treatments (15 days), the positive control had 100% eradication of the trophonts irrespective of their reduced concentrations and duration of treatment exposure, whereas, in the extracts group only group F (350mg/L) and group G (450mg/L) recorded a higher eradication of trophonts compared with the lower concentrations of 15mg/L in group D and 25mg/L in group E. Significant difference ($P>0.05$) was observed in day 15 among all the treated groups. In the gills

of the infested fish, it was observed that the degree of curative potentials of the extracts in comparison with standard drug in eradicating trophonts varied when compared to the level of the positive eradication of the trophonts from the body smear by the same concentrations of the extracts and standard drug. Moreso, there was significantly ($P<0.05$) low removal of the trophonts from the gills especially among the extracts treated groups where lower extracts concentrations (group D and group E) provided a higher removal of the adult stage parasites compared to the highest extracts concentration in group F (35mg/L) and group G (45 mg/L) in day 7. But significant differences ($P<0.05$) was observed in day 7 among the treated groups. However, the positive control (standard drug) group showed a reduced level of trophonts removal when compared to group D which had the highest percentage curative level as shown in Table 15. In day 15, there were a contrasts among the experimental groups especially among the treated groups in removal of the adult parasites from the gill of the infested fish where the positive control had the highest percentage cure, followed by group F, group G, group E and group D with mean values of 90.77±10.10%, 76.33±13.94%, 70.05±14.52% and 64.27±17.05%), respectively. In the gill of the infested fish, there was a significant $P>0.05$) percentage cure (in the treated groups, though no 100% removal of the parasites were observed in any treated groups.

Table 15: Mean percentage cure of aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) on the *Clarias gariepinus* infested with *Ichthyophthirius multifilis* parasites during prolonged bath treatments.

Experimental Group	Percentage (%) cure Day 7 (Body smear)	Percentage (%) cure day 15 (Body smear)	Percentage (%) cure Day 7 (Gill)	Percentage (%) cure day 15 (Gill)
A (Healthy control)	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 00^{a1}	0.00 ± 0.00^{a1}
B(infested not treated)	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}
C (standard drug)	88.78 ± 3.16^{c1}	100.10 ± 0.00^{c2}	61.87 ± 33.02^{b3}	$90.77\pm10.10^{b1,2}$
D(15 mg/L)	71.22 ± 14.92^{bc1}	$82.77\pm15,18^{b2}$	65.03 ± 7.27^{b3}	64.27 ± 17.05^{b3}
E (25 mg/L)	66.47 ± 20.72^{b1}	80.11 ± 4.86^{b2}	53.96 ± 19.25^{b3}	$70.05\pm14.52^{b4,1}$
F (35 mg/L)	$77.67\pm8.25b^{c1}$	89.73 ± 17.78^{bc2}	30.93 ± 36.64^{ab3}	$76.33\pm13.94^{bc4,1}$
G (45 mg/L)	71.93 ± 2.05^{bc1}	86.37 ± 2.17^{bc2}	37.53 ± 32.55^{b3}	72.37 ± 4.78^{bc1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).

3.4. Antiprotozoal activity of aqueous extracts of *Moringa oleifera* leaves on the key life stage (theront) of *Ichthyophthirius multifilis.*

Infective stage of the parasite was exposed to varied concentrations of aqueous *M. oleifera* extracts (B - G). The number of theronts surviving each concentration of the extracts displayed a clear dose response, survival decreasing with the increasing concentration of aqueous *M. oleifera* extracts used. The number of theronts surviving each concentration, given as the mean number of theronts that survived at the end of 24h of exposure were, 853.0±9.85, 771.67±49.36, 636.67±145.211, 346.00±97.26 and 121.33±60.14. The theronts were exposed to 00.1g/100ml, 0.02g/100ml, 0.03g/100ml, 0.04g/100ml and 0.08g/100ml concentrations, respectively. The percentage number of theronts that survived during the antiprotozoal activity of *M. oleifera* on the infective stage of the parasite, given as the mean percentage followed by the standard deviation were 94.77±1.14 for 0.01g/100ml; 85.73±5.49 for 0.02g/100ml; 70.70±16.15 for 0.03g/100ml; 38.43±10.80 for 0.05g/100ml and 37.45±42.56 for 0.08g/100ml after 24h exposure as shown in Table 16. The numbers that survived were significantly different ($P<0.05$). The level of mortality that occurred among theronts exposed to graded concentrations of aqueous *M. oleifera* extracts at the end of 24h was highest in the group treated with 0.08g/100ml of the extracts with mean value of 777.67±60.13, while the lowest level of mortality was recorded in group B treated with 0.01g/100ml of the extracts and with mean value of 47.0±9.84. Mortality was dose-dependent and was statistically significant at $P>0.05$. Moreso, no significant death was recorded in the group A (Negative control) when compared with other groups. The percentage death of the infective stage of the parasite exposed to the aqueous *M. oleifera* showed significance difference ($P<0.05$).

Meanwhile, the LC_{50} of the extracts was estimated at 95% confidence limit for concentration as 0.247g/ml (Fig. 4). Meanwhile, the model explains the data at 70.0% i.e. the model is efficient.

Table 16: Antiprotozoal activity of aqueous extracts of *Moringa oleifera* leaves on the key life stage (theront) of *Ichthyophthirius multifilis*

Experimental group	Mean no of theronts per replicate ~ 900 theronts per ml	No of theronts dead at the end of 24h	No of theronts survived	Percentage dead (%)	Percentage survived (%)
A (0.0g/100ml) of extracts	819.10±16.09[a]	4.00±4.34[a]	896.00±4.35[d]	0.43±0.49[a]	99.52±0.45[b]
B (0.01g/100ml)	855.67±16.04[b]	47.0±9.84[a]	853.0±9.85[d]	5.17±1.09[a]	94.767±1.14[b]
C (0.02g/100ml)	857.33±4.73[b]	128.33±49.36[ab]	771.67±49.36[ab]	14.23±5.45[ab]	85.73±5.48[b]
D (0.03g/100ml)	856.67±8.96b	263.33±145.21[b]	636.87±145.211[c]	29.26±16.13[b]	70.70±16.15[ab]
E (0.05g/100ml)	853.33±18.88[b]	554.0±97.26[c]	346.00±97.26[b]	61.52±10.79[c]	38.43±10.80[a]
F (0.08g/100ml)	845.0±8.54[b]	778.67±60.13[d]	121.33±60.14[a]	86.50±6.66[d]	37.45±42.56[a]

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different alphabets as superscript in a column are significantly different (P<0.05).

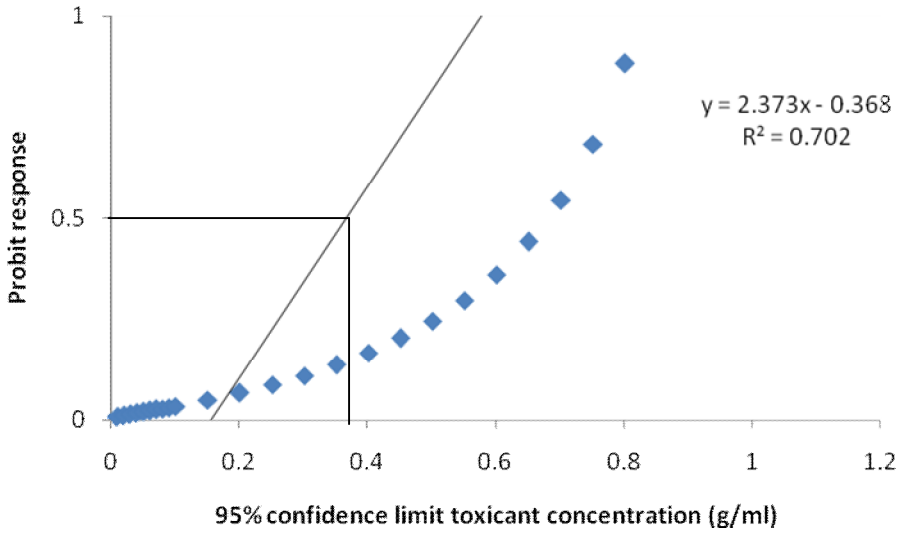

Fig 4. Probit transformed responses for minimum inhibitory concentration in the antiprotozoal activity of *M. oleifera* on the key life stage (theronts) of *Ichthyophthirius multifilis*

3.5. Morphological Alteration of Theronts Exposed to Different Concentrations of Aqueous Extractss of *Moringa oleifera* Leaves

The number of theronts that survived during the antiprotozoal activity especially in group F were very sluggish and appeared dead. The theronts gradually became spherical in shape, with loss of cilia after exposure to the extracts. During the deformation process, the theronts were alive at the early stage (Fig 5) and rotated at the same location and the treated theronts lost their swimming ability especially the cilia and died with a swollen body configuration/or deformation. The untreated live theronts (negative control) were dolicomorphic having a fusiform shape and well defined cilia as shown in Fig. 6.

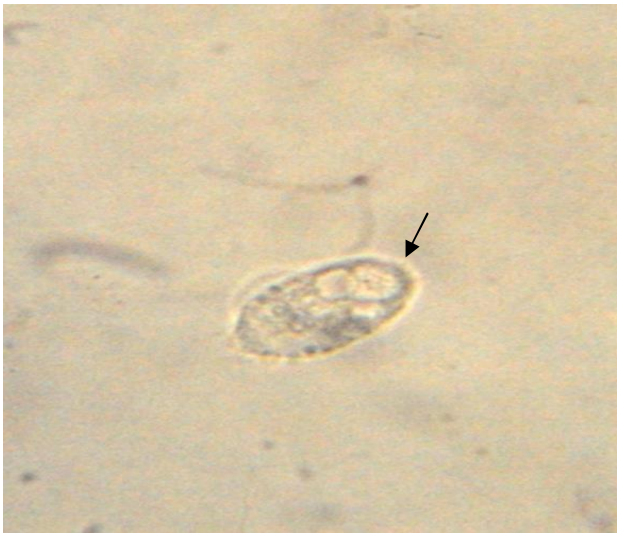

Figure 5. Normal morphology of theronts showing intact cilia (black arrow) with fusiform shape. Neutral red stain. mag. X40.

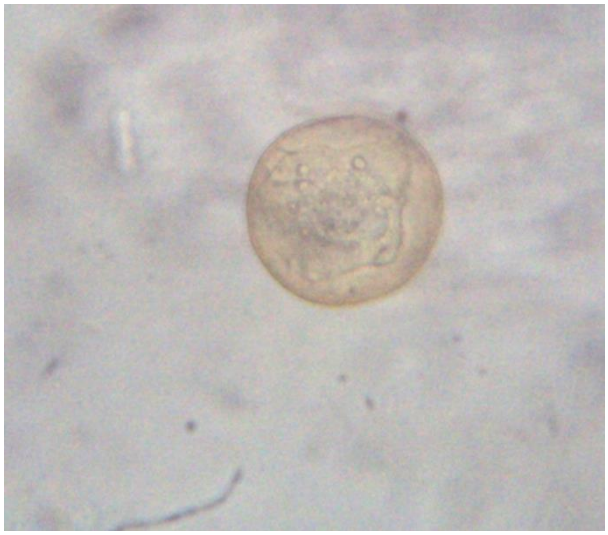

Figure 6. Deformed theronts after exposure to aqueous extracts of *Moringa oleifera* showing distorted and degenerated cilia and spherical shaped body. Neutral red stain. mag. X40.

3.6. Viability of Infectivity of Theronts after Pretreatment with *Moringa oleifera* Leaves Extractss for 1h and Exposed to *Clarias gariepinus* for 14 days

The infectivity of the theronts on *C. gariepinus* was lost or decreased appreciably after live theronts were treated with *M. oleifera* for 1h. Total number of approximately 7000 theronts were distributed into two different containers (A and B) group B pretreated with 0.5g/L of the extracts for 1h and allowed to reinfect normal healthy fish lost its infestation. Not all the pretreated theronts were able to penetrate the skin of the healthy fish. The number of trophonts that emerged in group B reduced drastically when compared to the group A (negative control) as shown in Table 16. There was a significant difference ($P<0.05$) between the negative control and the pretreated group when subjected to simple t-test. However, the emergence of white spots between the infested fish in group A and the pretreated group B differed significantly ($P<0.05$). For example, the number of white spots that emerged in the healthy fish was 607.80±289.62 while group B had 131.200±49.33. However, the number of trophonts present in the gill of fish exposed in group A and B showed significance differences ($P<0.05$) with the group A (negative control) having the higher number of trophonts as (9.40±4.16), whereas, the group B had the lower number of trophonts as (1.40±1.34). It can be deduced that the viability of the infective theronts when pretreated, might be reduced which led to the significant reduction in the number of trophonts in the gills, body smear and white spots decreased in group B in comparison to group A.

Table 16. Changes in the viability of infective theronts pretreated with 0.5g/L of aqueous extracts of *Moringa oleifera* leaves for 1h in infectivity potential in healthy *Clarias gariepinus*

Experimental group	Number of trophonts in the body smear	Number of white spots observed	Number of trophonts in the gill
A (Negative control)	76.80±30.14	607.800±289.62	9.40±4.16
B (0.5g/L) pretreated group	15.80±9.63	131.200±49.33	1.40±1.34

3.7. Infective Prevalence and Intensity of Theronts Pretreated for 1h with 0.5g/L of aqueous extractss of *Moringa oleifera* leaves

The infective prevalence and intensity of the theronts pretreated for 1h with 0.5g/L of *M. oleifera* decreased significantly ($P<0.05$) between the two groups (A and B). The infective prevalence of the group was the higher with mean value of 100.00±0.00 compared to the group B which had the lower infective prevalence of 31.10±25.25. Meanwhile, the infective intensity of 159.67±34.4 was recorded in the group A (negative control) in comparison with 82.17±30.99 recorded in the group A. The treatments of theronts with *M. oleifera* at 0.5g/L showed low infectivity prevalence and intensity (Table 17).

Table 18: Infective prevalence and intensity of theronts pretreated for 1h with aqueous extracts of *Moringa oleifera* leaves

Treatment groups	Number of theronts per replicate	No of fish per replicate n=15	Number of fish infested per replicate	Infestive Prevalence (%)	Infestive intensity
A(non-extracts treated)	å 7,000	5 5 5	5 5 5	100 100 100 (100±0.00)	120.8 184.2 174.8 (159.67±34.14)
B (0.5g/L of extracts)	å the 7,000	5 5 5	2 3 1	13.3 20 60 (31.10±25.25)	105.5 47 94 (82.17±30.99)

3.8. Gross Morphological Examination of *Clarias gariepinus Infested with Ichthyophthirius multifilis* under Stereomicroscope

In the current study, all infected fish showed white nodules on the body Fig. 7, eye (Fig. 8) and some of them showed lethargy and increased mucus production. The ulceration or erosion as seen in Figure 9 resulted from the entrance of parasites into skin and exit from the host are probably damaging to its feeding activity while it was on the host. In the gills, there was prominent change in the colour from red to pale colour (Fig. 10) due to the accumulation of the parasites in the gill which possibly affected respiration.

Figure 7. Fish (*Clarias gariepinus*) exhibiting white spots (white arrow) on the body depicting Ichthyophthiriasis disease. Mag. 20X.

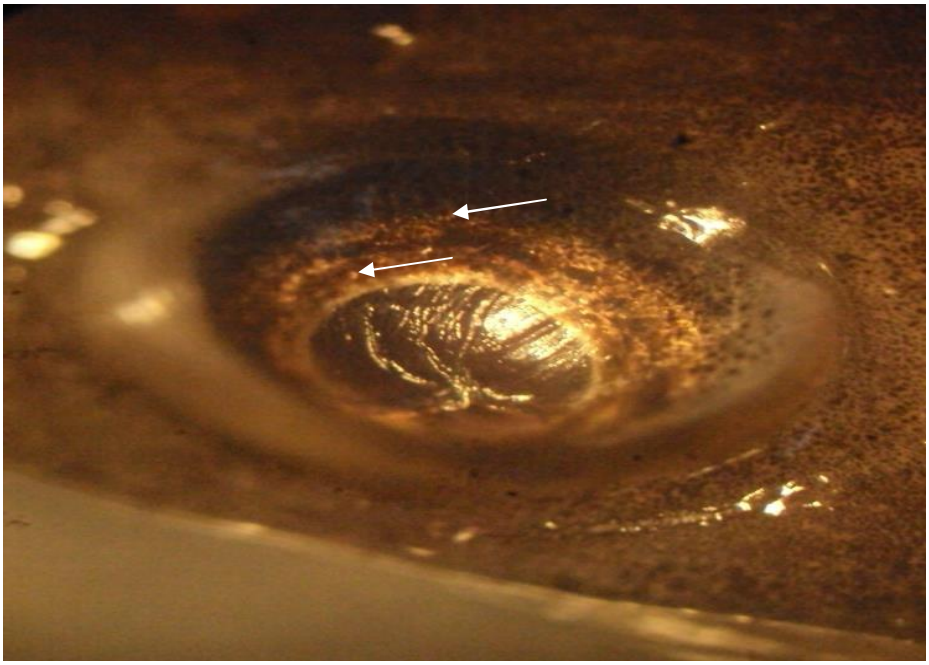

Figure 8. Localisation of white spots at the eye region (white arrow) of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* under steromicroscope. Mag. X20

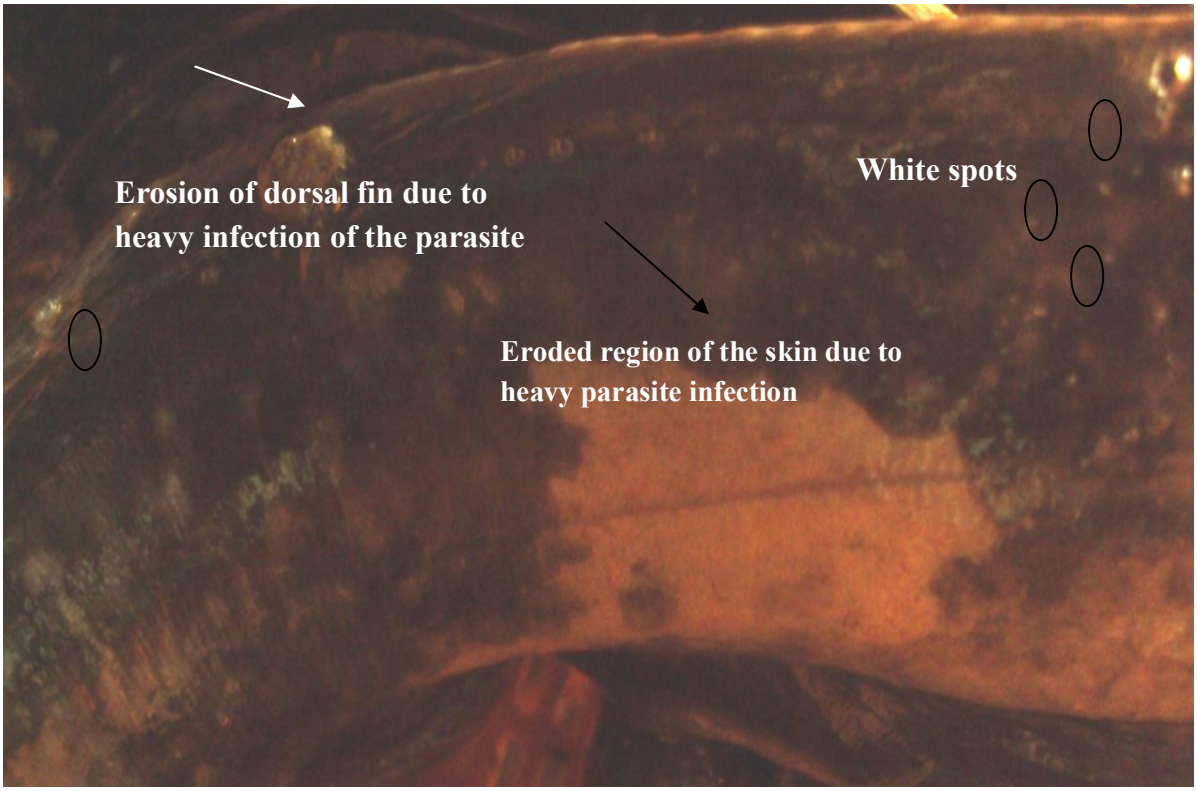

Figure 9. Gross morphology of infected Fish showing white spots (circles) and erosion of the skin (black arrow) and dorsal fin (white arrow) due to Ich parasite. Mag. 20X.

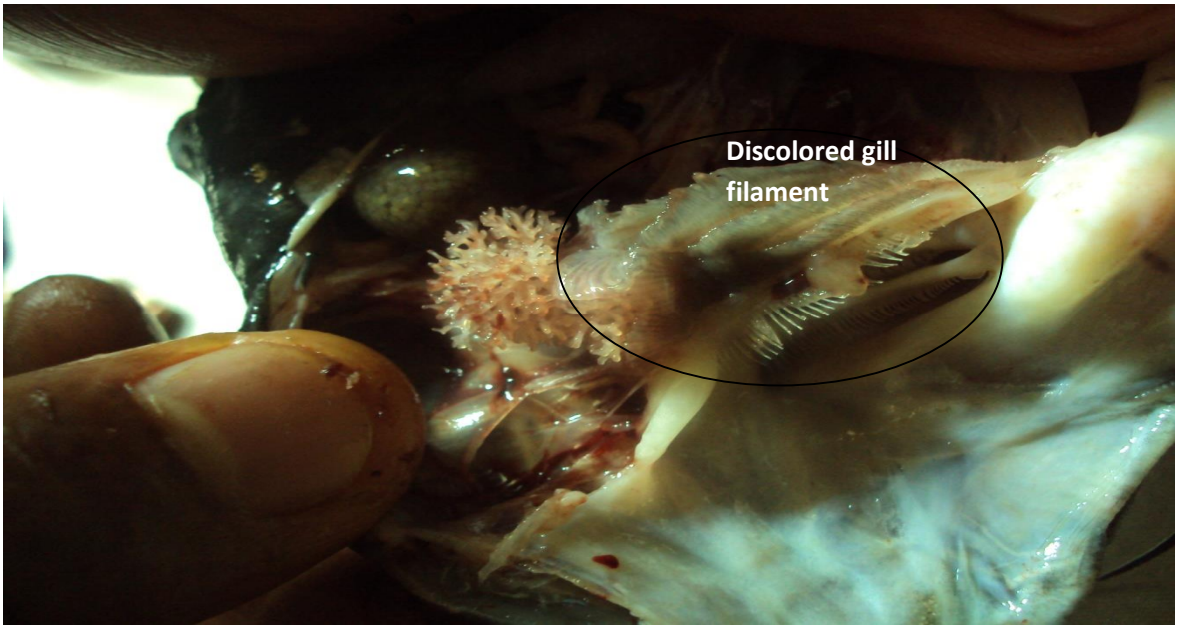

Figure 10. Infected fish showing prominent change in the color of the gill structure especially the gill filament due to parasite invasion.

3.9. Histopathology of *Clarias garepinus* Skin Infested with *Ichthyophthirius multifilis* During Dip, Short term and Prolonged bath Treatments (1h) with aqueous extractss of *Moringa oleifera* Leaves and Standard Drug (fish cure).

3.9.1. Histopathology of *Clarias garepinus* skin infested with *I. multifilis* during dip treatments (1h) with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

The skin of the control during 1h dip treatments showed normal skin morphology such as chromatophores, collagen cells which is a major structural protein in the skin (Plate 1) was evident. The skin of *C. garepius* after being exposed to infective theronts before treatment showed severe damage such as; presence of inflammatory cells, degeneration of fats (micro and macro vesicular) and heamorrhaging due to arrays of red blood cells (Plate 2). The aberrant conditions of skin tissue of *C. gariepinus* infested with *I. multifilis* and not treated includes; abscess formation, vacuolation, and shrinking of the muscle fibre as shown in Plate 3. The group treated with standard drug exhibited positive response in the restoring the skin architecture with intact epidermal and dermal layers of the skin (Plate 4). The groups treated with aqueous extracts of *M. oleifera* (group D and G) showed that the muscle layers were affected by the loss of their normal morphology. These appeared as elongated masses of unidentified tissue without any trace of nuclei (Plate 5). Skin was observed with large spaces between deeper tissues, bundles of fibres fused together leaving small spaces and exhibit hyaline degeneration (Plate 6 and 7) in the groupôs treatment with 2,500mg/L and 3,500mg/L. However, shrinkage and abscess formation (Plate 7) fragmentation and vacuolation (Plate 8) were evident in the groups treated with 3,500 mg/L and (group F) and 4,500 mg/L (group G), respectively.

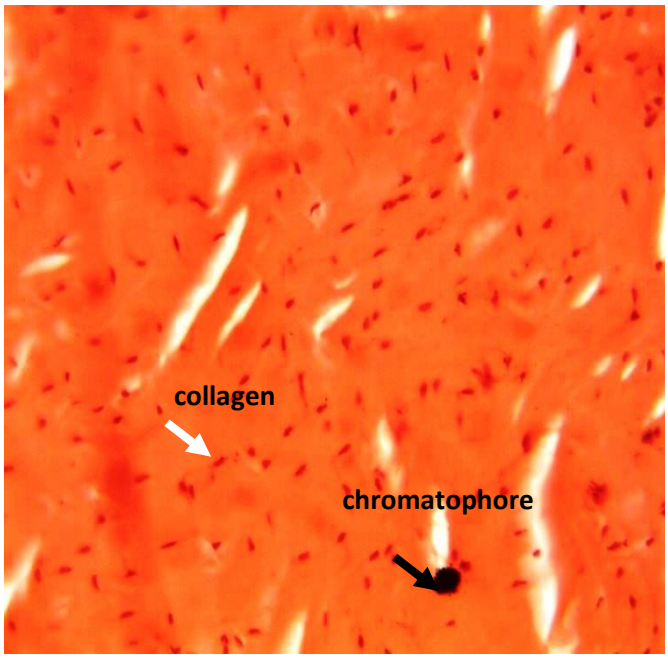

Plate 1. Skin of control group showing normal skin architecture; chromatophore (black arrow) is a pigment-containing cell (melanin)in the deeper layers of the skin and collagen (white arrow) which is major structural protein in the skin which plays a positive role in providing structural scaffolding, strength and elasticity in the skin. H&E Mag x100.

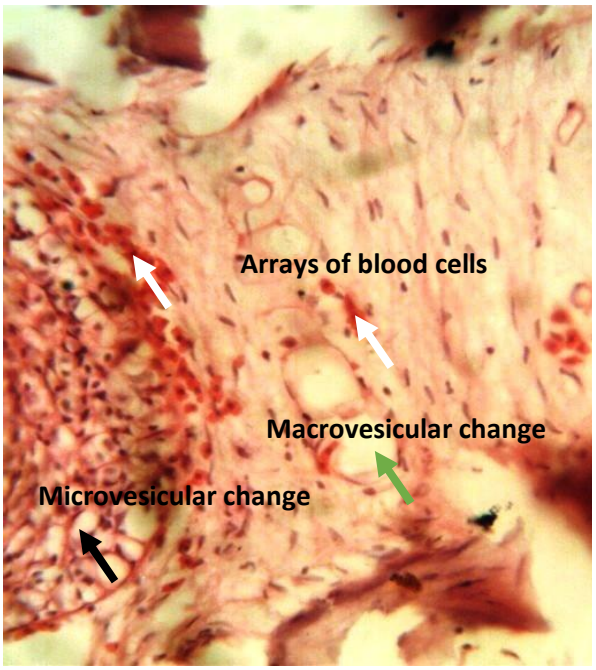

Plate 2: Photomicrograph of the skin (pigmented) of *Clarias gariepinus* infected with Ich before treatment with aqueous extracts of *Moringa oleifera* leaves. There is micro (black arrow) and macro vesicular (green arrow) fatty degeneration, with arrays of blood cells (white arrow). H&E. mag. 400x.

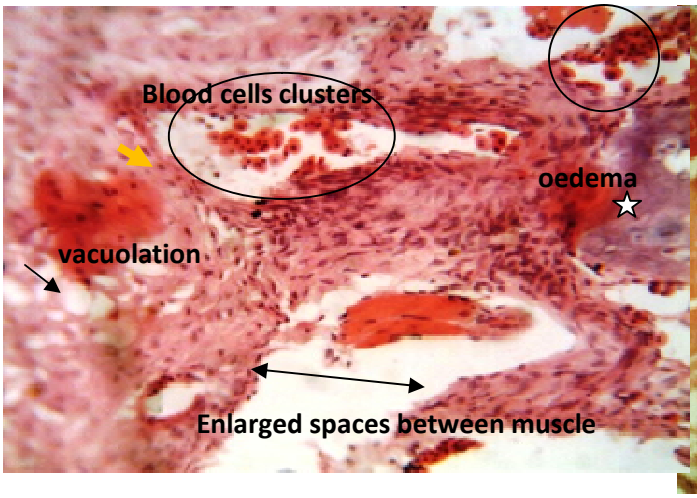

Plate 3: Photomicrograph of the skin (non pigmented) of *Clarias gariepinus* (group B-infected not treated) infested with *Ichthyophthirius multifilis* after dip treatment (1h). Note the penetration of inflammatory cells (yellow arrow), blood cells clusters (circles), Abscess formation (star), vacuolation (black arrow) and enlarged spaces between the muscle fibre due to shrinkage of muscle fibre. This depicts tissuedamageH&E.mag100x.

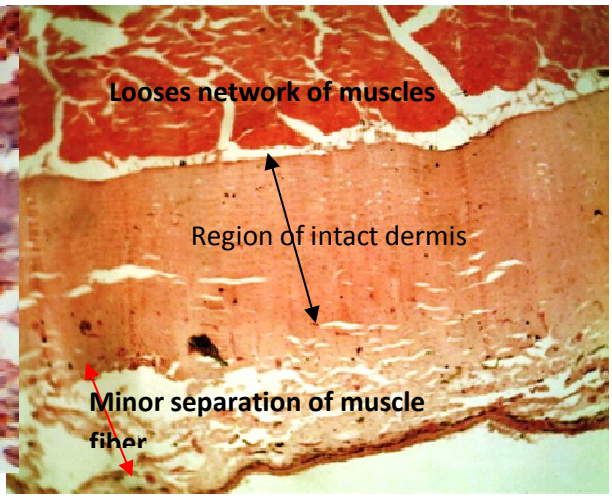

Plate 4: Photomicrograph of fish skin of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* and treated with standard drug (fish cure) (Group C) at the end of the treatment period (1h). There is no prominent damage in the skin tissue. Minor separation of muscle fibre (red arrow head), intact zone of dermis (black arrow head) and pigments were observed. The epidermal basal layer is intact. H&E. Mag. 100X.

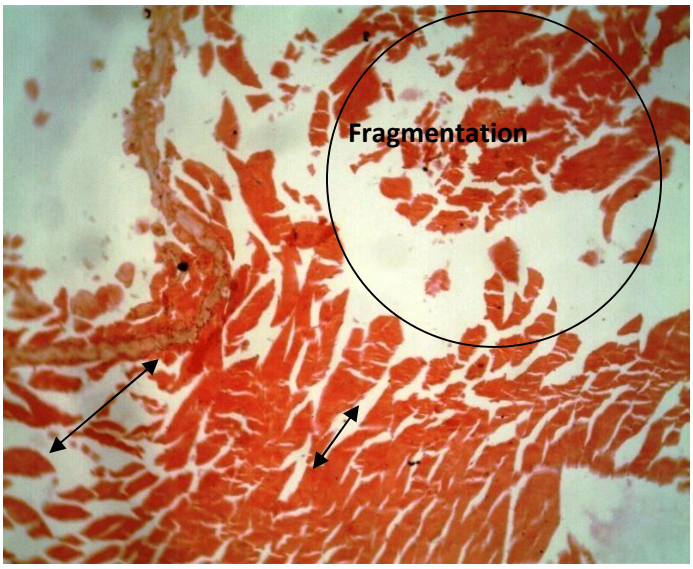

Plate 5: Photomicrograph of Group D skin (non-pigmented) off *Clarias gariepinu* infested with *Ichthyophthirius multifilis* and treated witth 1500mg/L aqueou extracts of *Moringa oleifera* leaves showed Severe fragmentation (circles) an separation (black arrow) of the muscle fibre were observed. H&E. mag. 400X.

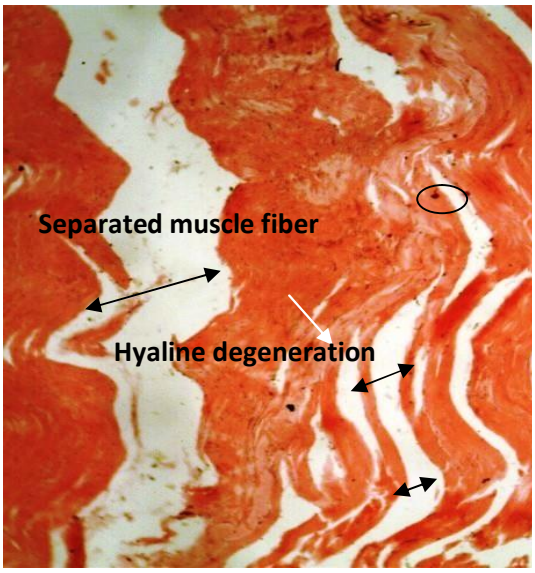

Plate 6: Photomicrograph of group E skin of *C. gariepinus* infected with *Ichthyophthirius multifilis* and treated with 2500mg/L aqueous extracts of *Moringa oleifera* leaves showed shrinkage of muscle fibres resulting in the separation of muscle fibres (black arrow) with few muscle fibre nuclei (circles) and hyaline degeneration (whitearrow). H&E.mag.400X.

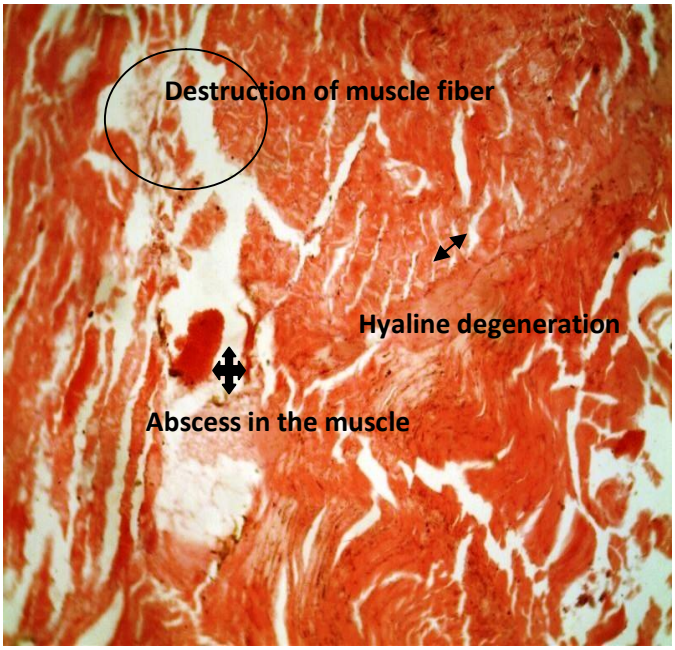

Plate 7: Photomicrograph of group F skin of *Clarias gariepinus* infected with Ich and treated with 3500mg/L aqueous extracts of *Moringa oleifera* leaves showed There is severe infection and damage of the skin due to presence of abscess in th muscle (+), hyaline degeneration (black arrow) and destruction of the muscle fibre (circles).H&E. mag 100X.

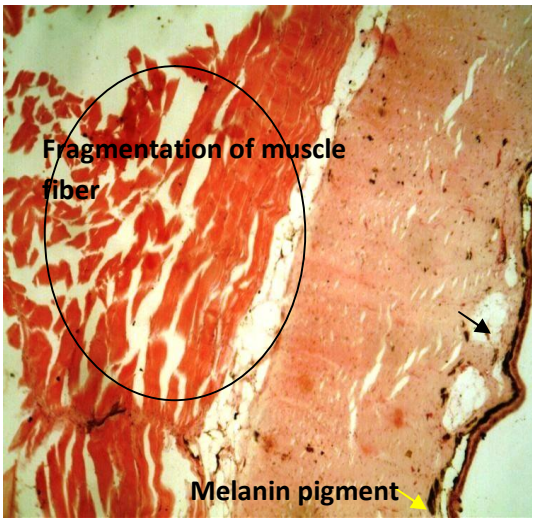

Plate 8: Photomicrograph of group G skin of *Clarias gariepinus* infected with Ich and treated with 4500mg/L of aqueous extracts of *Moringa oleifera* leaves showed. Skin (pigmented), due to presence of melanin which forms a straight line along the basal membrane of the skin. Vacuolaton in dermis (black arrow) and fragmentation of the muscle fibre were observed. This is keeping for possible skin damage due to parasite invasion in the skin. H&E. mag 100X.

3.9.2. Histopathology of skin of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* after short-term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Histopathological observations during the short term treatment period showed that normal control (group A) exhibited intact muscle fish, mucus cells and melanophores which comprises of the normal structural parts seen in *C. gariepinus* skin (Plate 9). Changes in the histology of skin was seen among *C. gariepinus* infected with *I. multifilis* before treatmentwhich showed skin dermatitis, multiple muscular abscess and disintegration of the muscle fibre (Plate 10).

At the end of the 96hr exposure (short-term treatment), group B (infested not treated) showed high degree of skin damages such as enlargement of the mucous cells, oedema and severe abscess in the skin (Plate 11). The skin of *C. gariepinus* infected with ichthyophthiriasis and treated with standard drug showed mild degree of inflammation at the superficial layers of the skin (Plate 12) and intact collagen and elastic fibres. At the same time, erosion of the outer surface of the skin was observed with large spaces (atrophied cystic spaces) with multiple lesion which led to dissolution of the tissue, high influx of inflammatory cells (Plate 13) in the group D treated with 150mg/L of aqueous extracts of *M. oleifera* leaves were evident.

The group E treated with 200mg/L aqueous extracts of *M. oleifera* at the end of 96hr of exposure, showed that the skin of *C. gariepinus* infested with *I. multifilis* did not show severe damage but only separation of muscle fibres with nuclei and large spaces forming in between the muscle fibres (Plate 14). Few muscle fibres were present here and there while the large part of

the skin showed several rounded and elongated spaces and in some of these areas, tissues were undistinguishable. Severe fragmentation in the muscle fibre (Plate 15) was observed in the skin of the test animal which was treated with 350mg/L of the extracts. Similar findings was also observed in the skin of *C. gariepinus* treated with 450mg/L of the extracts such as separation/distortion of muscle fibres (Plate 16) while freckle was also observed due to the aggregation of pigment at particular spot. The pathological changes due to the administered treatments in regulating the white spot appeared reduced especially among the standard drug treated group when compared to infected not treated, and infected treated with varied concentrations of *M. oleifera* extracts.

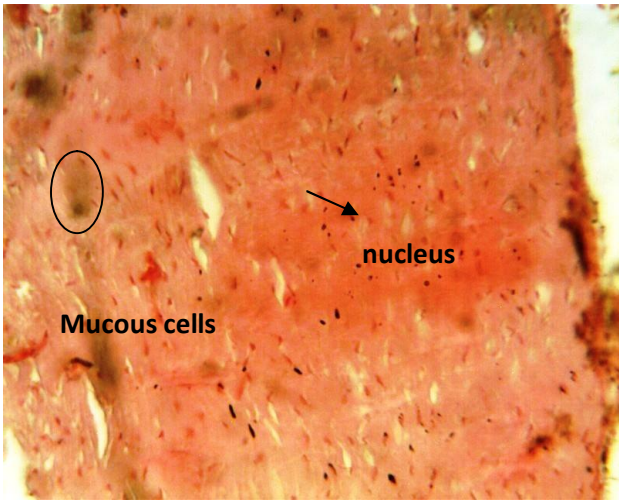

Plate 9: Photomicrograph of normal skin of *Clarias gariepinus* showing intact muscle fibre with numerous nuclei (black arrow) and mucous cells (circles) H&E. mag 100X.

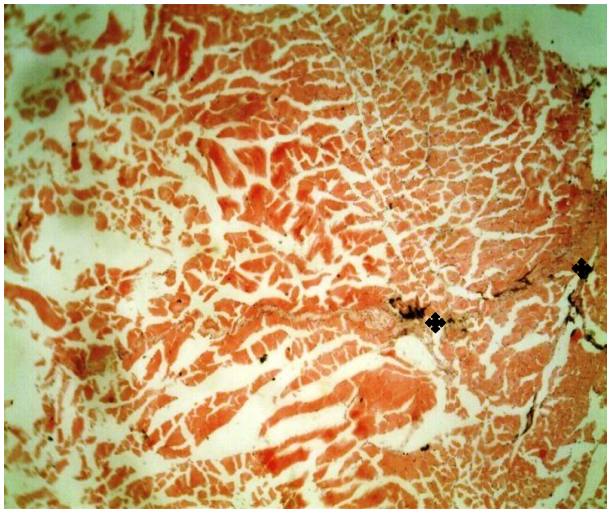

Plate 10: Photomicrograph of skin of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* before treatment. Severe skin (pigmented) dermatitis with multiple muscular abscess (+) around the muscle fibres and the muscle fibres are severely destroyed with numerous spaces in between the muscle. H&E. mag 400X

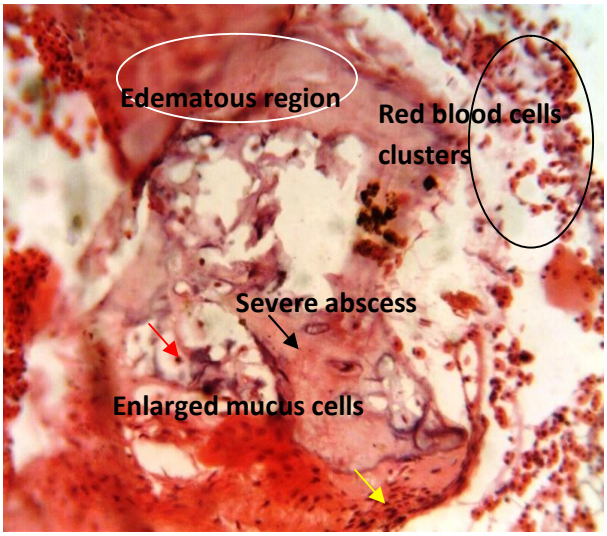

Plate 11: Photomicrograph of group B (infected not treated) skin of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* after 96h of exposure. Severe skin damage was observed due to severe abscess (black arrow), enlarged mucous cells (red arrow), arrays/or clusters of blood cells (circles), proliferation of inflammatory cells (yellow arrow), skin appeared edematous (white circles and enlarged spaces (+) caused skin degeneration and deformation. H&E. mag 400X.

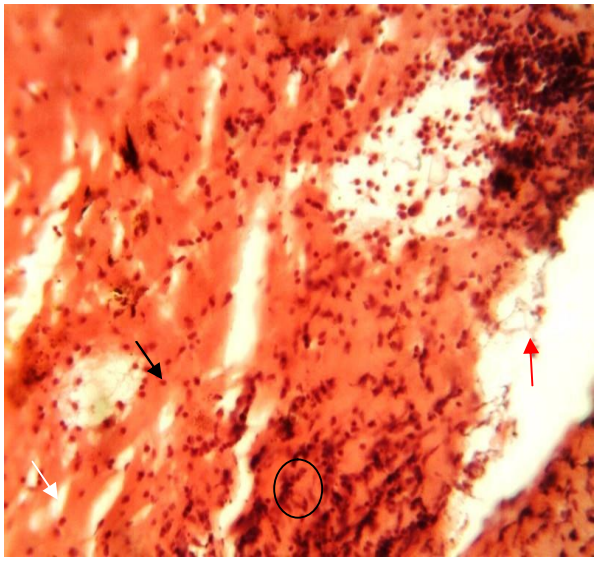

Plate 12: Photomicrograph of group C skin of *Clarias gariepinus* infected with *Ichthophthirius multifilis* and treated with standard drug (fish cure) after 96 h. There are clusters of inflammatory cells (black arrow) at the superficial layers of the skin. Some are already necrotic (circles). However collagen (white arrow) and elastic fibres (red arrow) are seen at the reticular layer of the skin. H&E. mag. 100X

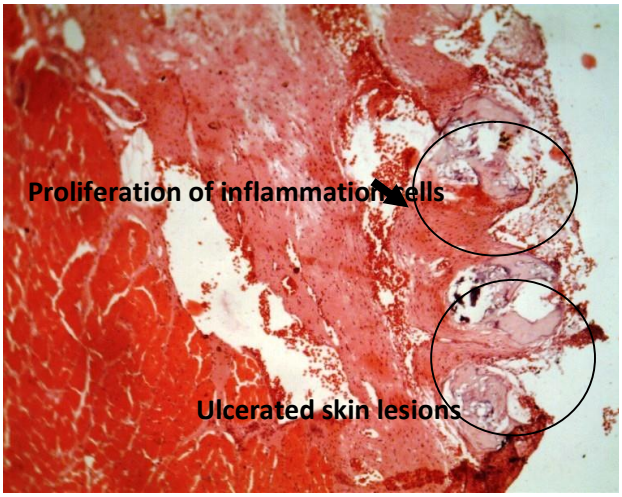

Plate 13: Photomicrograph of group D skin of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* and treated with 150mg/L of aqueous extracts of *Moringa oleifera* leaves after 96 h showed Proliferation of inflammatory cells (black arrow) with multiple skin lesions (circles). H&E. mag 100X.

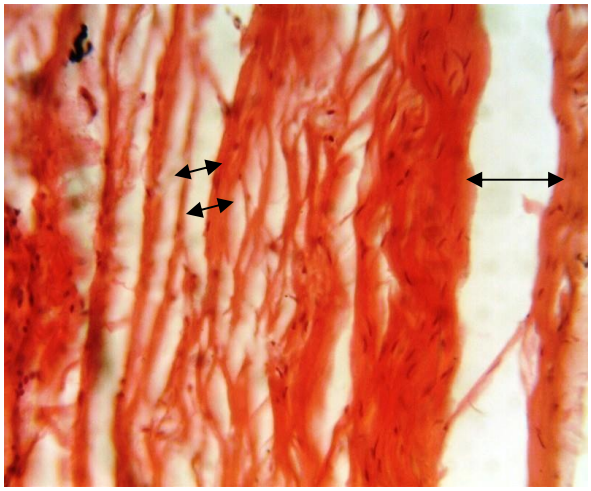

Plate 14: Photomicrograph of group E skin of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* and treated with 250mg/L aqueous extracts of *Moringa oleifera* after 96 h showed Separation of muscle fibres with few nuclei seen (black arrow). Note the spaces depict infection and damage to the skin. H&E. mag. 100X

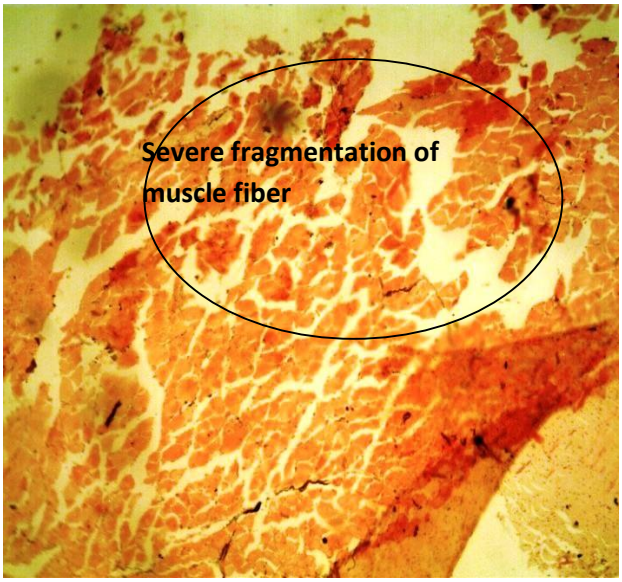

Plate 15: Photomicrograph of group F skin of *Clarias gariepinus* infested with *Ichthyophthirius. multifilis* and treated with 350mg/L aqueous extracts of *Moringa oleifera* leaves after 96 h. showed Severe fragmentation of muscle fibre (circles). H&E. mag 100X.

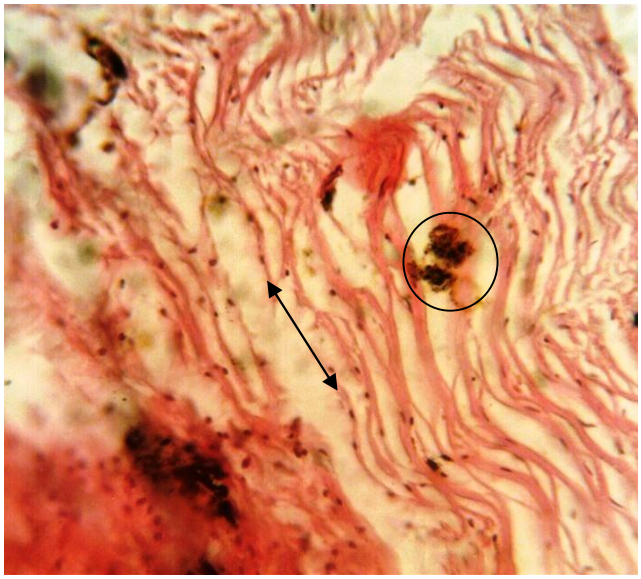

Plate 16: Photomicrograph of group G skin of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* and treated with 450mg/L aqueous extracts of *Moringa oleifera* leaves after 96 h showed Separation of muscle fibres with numerous nuclei attached to it (white arrow). Pigments are seen which are concentrated at a particular spot thereby forming freckle. However, the muscle fibre appears distorted (black arrow). H&E. mag. 100X.

3.9.3. Histopathology changes in *Clarias gariepinus* skin after prolonged bath treatments (5 - 15 days) with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Microscopically, the normal control showed intact myotomes alarm cells, with traces of melanin pigment as shown in Plate 17. Consistent changes observed in the skin of the *I. multifilis* infected fish were enlargement of the mucous cells (Plate 18), hypertrophy and densely thickened pigment. In the infected not treated skin, there were keratinization, edema and pyknotic nuclei (Plate 19). There is an intact muscle fibre at the superficial layers of the skin which appears highly pigmented (Plate 20). The skin at the same time showed clogging up of the muscle fibres which appeared aggregated with blood cells due to heamorrhage (Plate 21), muscle fibres appears degenerated and densely pigmented (Plate 22). Influx of blood cells in the skin of the infested test animal was evident and caused possible petichiation (Plate 23) at the dermal layer of the skin especially in the group treated with 15mg/L and 35mg/L of the extracts. The texture of the skin showed aberrant conditions due to the penetration of protozoanôs parasites from the surface to deeper layers thereby disrupting the normal architecture of epidermis and dermis (Plate 24). At the same time, there are large parts of the skin that exhibited several rounded and elongated spaces and in some of these areas, tissues were indistinguishable. The degree of pathological changes varied among the extracts treated group, standard drug and the control groups.

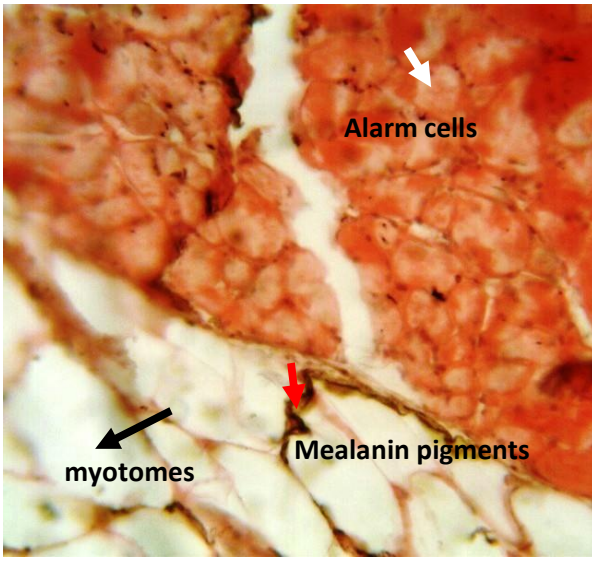

Plate 17: Normal control showed pigmented skin (blue arrow), intact myotomes (black arrow), alarm cells (white arrow) melanin pigments (red arrow) all keeps for normal morphology of skin H&E. Mag. 400X

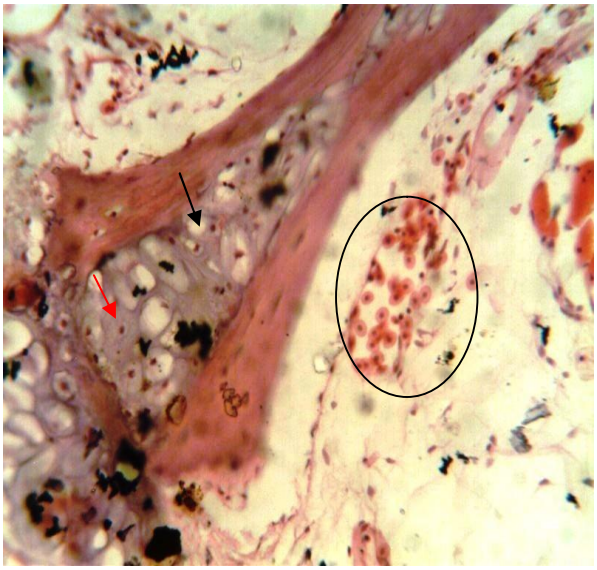

Plate 18: Photomicrograph of skin of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* before treatment. Typical skin showing sebaceous gland with clusters of cells hypertrophying (enlarged mucous cells) (black arrow) and epithelial cells-melanocytes (red arrow) arrow). However, the skin pigments are densely thickened and are surrounded at different spots of the skin forming what is called a freckle. H&E. 400 X.

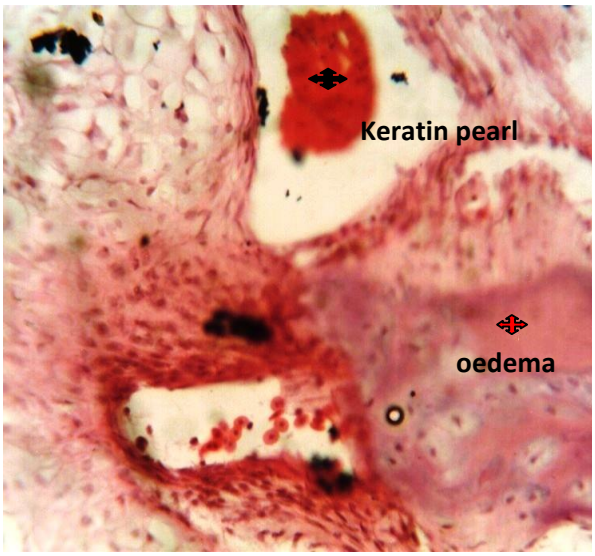

Plate 19: Photomicrograph of group B (infected not treated) skin of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* after 15days of exposure. Cells appear focally differentiating and has become flattened and pyknotic (circles) and fully keratinizing (+), forming a keratin pearl (+) and edema (red cross). H&E. mag 400X.

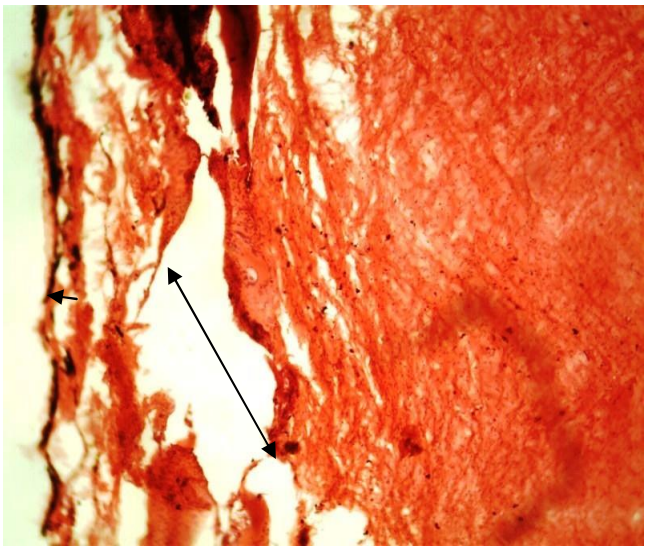

Plate 20: Photomicrograph of group C skin of *Clarias gariepinus* infected with *Icthyophthirius. multifilis and* treated with standard drug after 15 days of exposure. The superficial layer of the muscle fibres are Intact but with a little separation of the muscle fibre (double arrow) at the reticular layer of the skin. However the skin is also pigmented (black arrow). H&E. mag 100X.

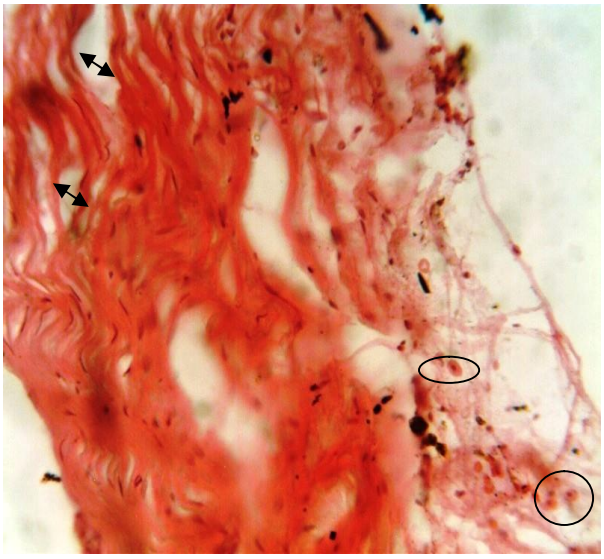

Plate 21: Photomicrograph of group D skin of *Clarias gariepinus* infected with *Ichthyopthirius multifilis and* treated with 15mg/L aqaeous extracts of *Moringa oleifera* after 15days of exposure showed a quite progressive response to the extracts due to the clogging up the muscle fibre at the deep layer of the skin (double arrow). Moreso, the blood cells are aggregated at the surface of the skin due to heamorrhages in the skin (circles). H&E. mag 400X.

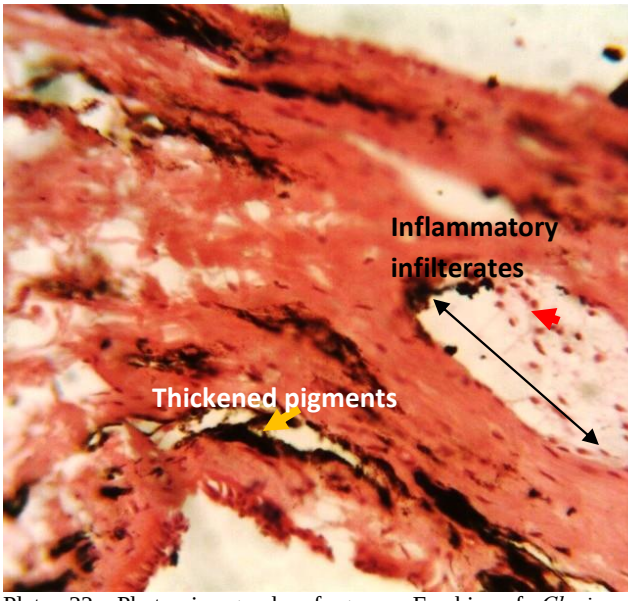

Plate 22: Photomicrograph of group E skin of *Clarias gariepinus* infected with i*chthyophthirius. multifilis and* treated with 25mg/L aqueous extracts of *Moringa oleifera* after 15days of exposure showed densely pigmented skin (yellow arrow) with degeneration of muscle fibre (double black arrow) with penetration of inflammatory cells (red arrow). H&E. mag. 400X.

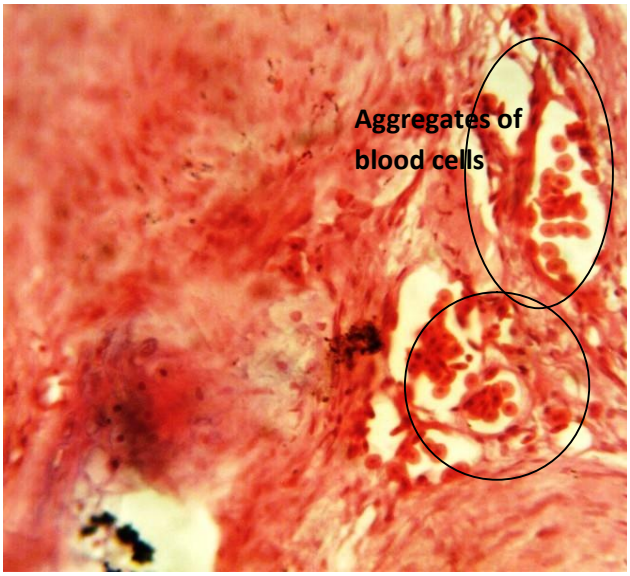

Plate 23: Photomicrograph of group F skin of *Clarias gariepinus* infected with *Ichthyophthirius multifilis and* treated with 35mg/L aqueous extracts of *Moringa oleifera* after 15days of exposure showed influx of the blood cells keeping for possible petichiation at the dermal layer of the skin .H&E. mag. 400X

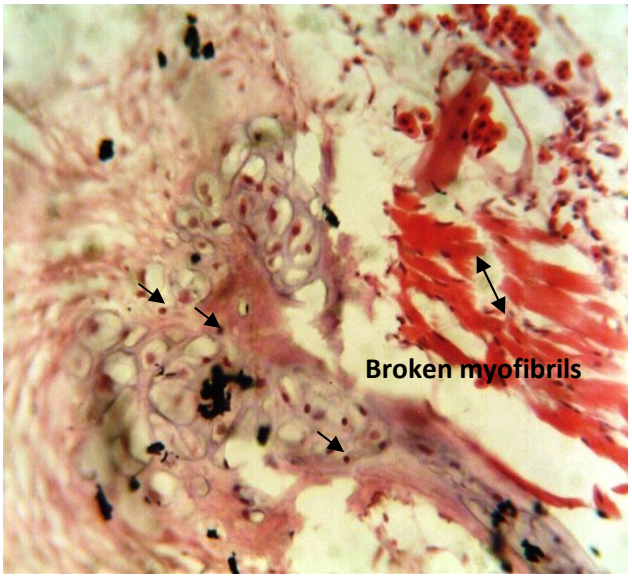

Plate 24: Photomicrograph of group G skin of *Clarias gariepinus* infected with *Icthyophthirius multifilis and* treated with 45mg/L aqaeous extracts of *Moringa oleifera* after 15days of exposure showed the skin texture having aberrant conditions due to the penetration of the protozoan parasite (circles). However, there are clusters of melanocytes (dark arrow) and distortion of the muscle fibre. H&E. mag .400X

3.9.4. Histopathology of *Clarias garepinus* gill infested with *Ichthyophthirius multifilis* during dip treatments (1h) with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

The section obtained from normal control group revealed normal histological patterns (Plate 28). The gill was made up of filaments or primary lamellae, arranged in double rows along the bone. Secondary gill lamellae originate from the filaments and are disposed perpendicular to the inferior and superior margins of each filament. Each secondary lamella contains thin-walled gill sinusoids that allows for the continuous exchange of respiratory gases.

The gill of infested fish before treatment revealed only the tendency of some secondary lamellae being distorted/or eroded and distended with oedmatous fluid due to possible increase in intracellular vacuolation which signaled on set of oedematous changes and vasoconstriction of the central venus sinuses (Plate 25) Similarly, the infected untreated group had a proliferative and degenerative alteration of the gills which led to possible destruction and corruption response to the pathogen. In the gills of the infested untreated, mature adult trophonts (Plate 26) were observed at the base of primary filaments as well as within the gill epithelium. However, there were lots of inflammatory cells surrounding the position of the adult parasite in the gills.

The degree of multiple enlargements of the various venous sinuses at the end of 1h exposure in the group treated with standard drug was found to be moderate, whereas, high level of proliferation of leukocytic infilterates in the gill epithelium (Plate 27) was grossly reduced. The histopathological lesions observed in the group D treated with 1500mg/L aqueous extracts of *M. oleifera* showed lamellar fusion / or clubbing especially in the secondary lamellae due to a proliferation of the epithelial cells at the base and tips of the secondary lamellae. These cells are

usually larger and more densely stained with more abundant epithelial cells. Some areas of central venus sinuses appear to be vasoconstricted and dilatated (Plate 29). The gills of *C. gariepinus* in group E (2,500mg/L) had destroyed gill lamellae which appeared irregular in shape in multiple patterns (Plate 30) following a minor degree of leukocytes infiltration. Similarly, group F (3,500mg/L) treated groups also showed similar pattern of the distortion or disintegration of the secondary lamellae (Plate 31), whereas, the gill of the group G (4,500mg/L) treated group exhibited severe destruction of the secondary lamellae (Plate 32). Following exposure before and after treatment (1h) and was similar with significant variations in four concentrations (1,500mg/L - 4,500mg/L. This was because some of the lamellae were thin and elongated, particularly the secondary lamella. The histopathological changes observed during the dip treatments showed that the standard drug (positive control) was able to regulate the penetration of the parasite in the gill which was potentially harmful when compared to the groups (D - G) treated with varied concentrations of *M. oleifera* extracts and the negative control (infected not treated). It could be deduced that during the dip treatments that the standard drug could at a minimum time control, regulate and possibly eradicate invaded adults or infective stage of the parasite in the gill, of *C. gariepinus.*

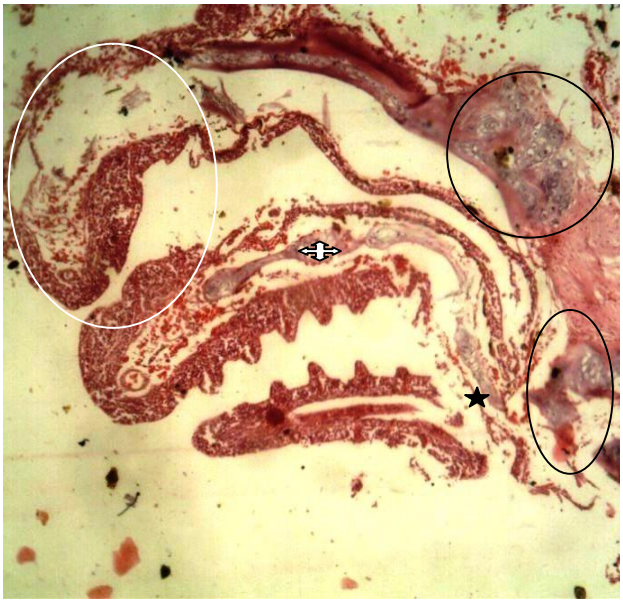

Plate 25: Gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* before treatment showed severe destruction of the gill especially the secondary lamellae which appears curled and destroyed (white circles), vaso constriction of the central venous sinus (+), oedema in the gill (black circles) and distortion or erosion of part of the secondary lamellae (*). H&E. mag. 400X.

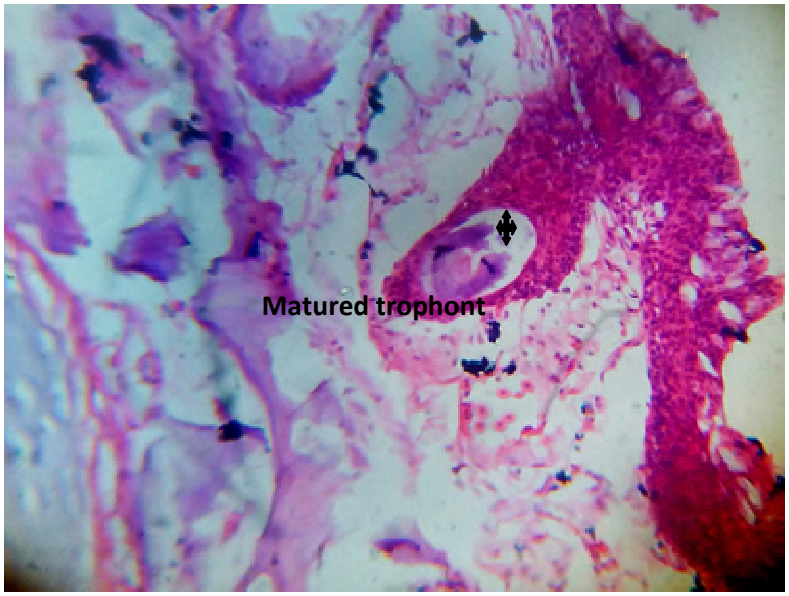

Plate 26: Gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* and no treatment was administered (Group B) at the end of 1h exposure showed presence of matured adult trophont embedded at the gill lamellae (+) with severe destruction of the gill architecture. However, blood cells were equally seen around the lamellae. H&E. mag. 400X.

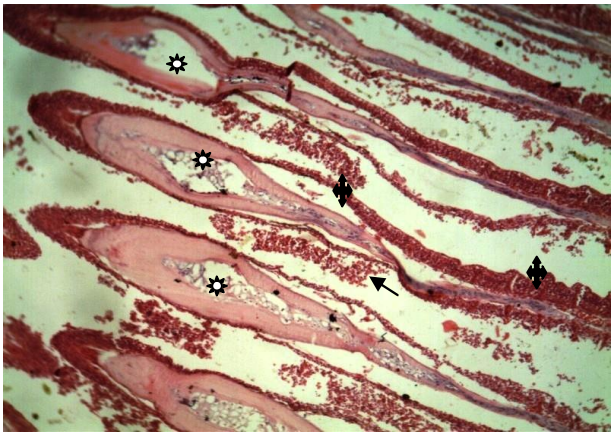

Plate 27: Gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* and treated with Standard drug at the end of 1h exposure showed multiple dilatation of the central venous sinuses (*) with loss of secondary lamellae architecture (+) with proliferation of leukocytic infilteration in the gill epithelium (arrow head) H&E. mag 400X.

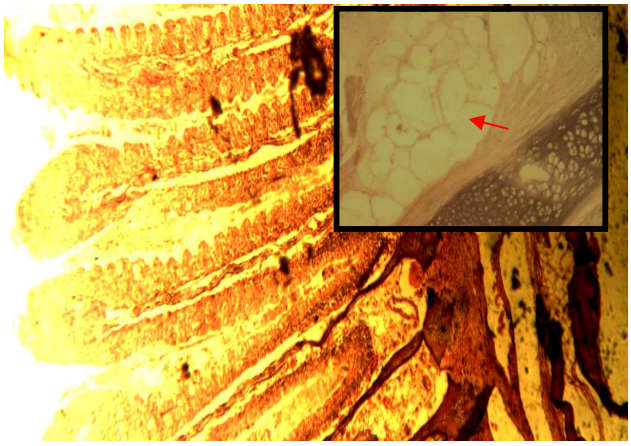

Plate 28: Normal control: Photomicrograph of gill structure showing in Group A: (1) normal gill structure with primary lamella (PL), secondary lamellae (SL) and central venous sinus (CVS), the elastic cartilage of the filament (black arrow) and efferent branchial arterioles (red arrow) were intact. H&E mag X100.

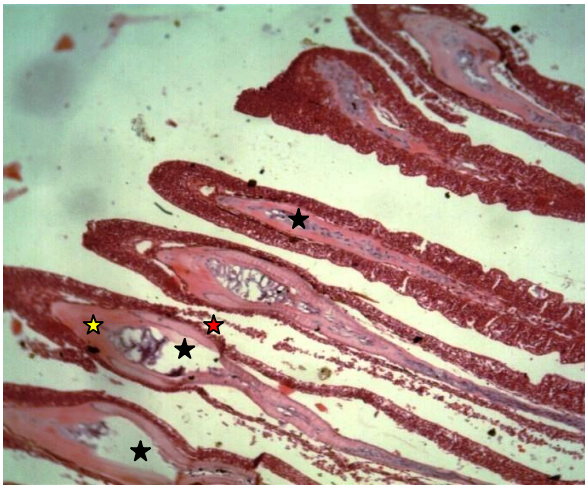

Plate 29: Gill of *Clarias gariepinus* in group D infested with *Ichthyophthirius multifilis* and treated with 1500mg/L aqueous extracts of *Moringa oleifera* at the end of 1h exposure showed vasoconstriction (yellow star) and vasodilatation (black star) of the central venous sinus, partial fusion of the tip of the secondary lamella (red star). H&E. mag. 400X.

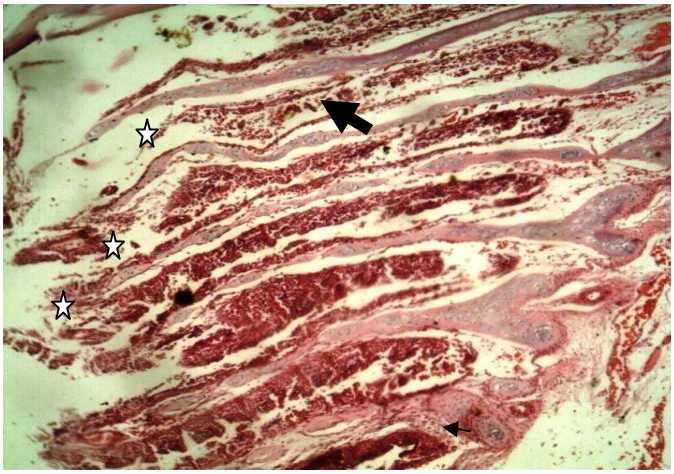

Plate 30: Gill of *Clarias gariepinus* in group E infected with *Ichthyophthirius multifilis* and treated with aqueous extracts of 2500mg/L *Moringa oleifera* severe destruction of the gill lamellae which appears to be irregular in shape(*) and infilteraton of leucocytic infilterates was seen (black arrow). H&E mag. 400X.

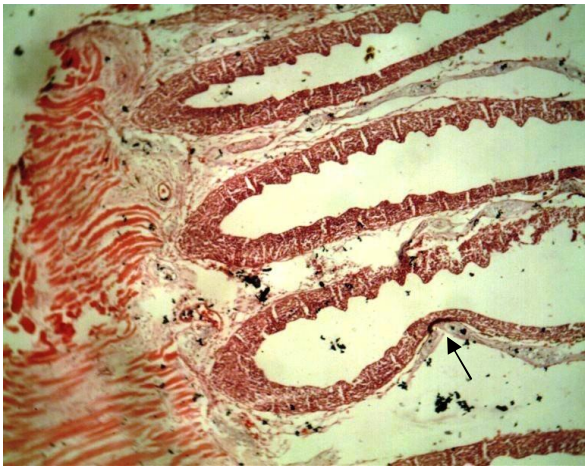

Plate 31: Gill of *Clarias gariepinus* in group F infested with *Ichthyophthirius multifilis* and treated with 3500mg/L aqueous extracts of *Moringa oleifera* showed distortion of the secondary lamella (black arrow). H&E. mag. 400X.

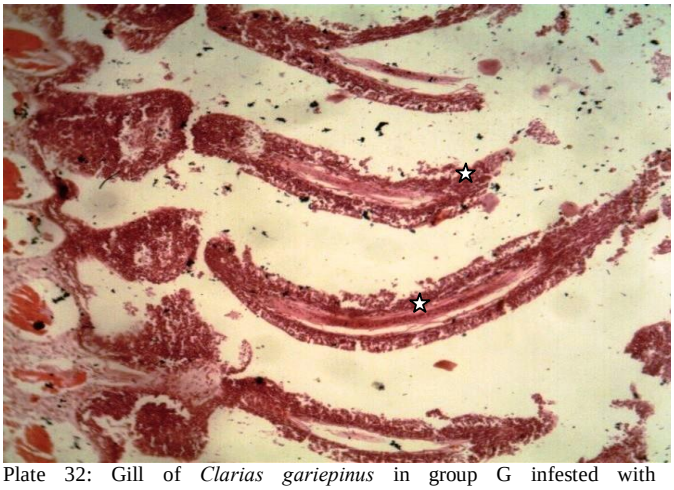

Plate 32: Gill of *Clarias gariepinus* in group G infested with *Ichthyophthirius multifilis* and treated with 4500mg/L aqueous extracts of *Moringa oleifera* showed epitheliocystis; note the basophilic ball like shape, different degree of secondary lamellae destruction is evident (*). H&E. mag. 400X.

3.9.5. Histopathological changes in the gills of *Clarias gariepinus* exposed to short term bath treatments (24 - 96h) with aqueous extracts of *Moringa oleifera* leaves extracts and standard drug (fish cure).

The result of the light microscopic (Lm) examination of the photomicrograph of the vertical section of the normal gills of *C. garipeinus* (Plate 28) showed the arrangement of the lamella. The primary lamella is rounded at the apices while the projecting secondary lamellae are clearly interspaced. At the end of the infection cycle, before treatment, the gills showed a high level of increased intracellular vacuolation which depicts oedema, fusion of the gill lamellae and increased blood flow inside the lamella which caused blood congestion or aneurysm (Plate 33). However, aggregation of red blood cells was observed in infected not treated group which depicts hemorrhaging in the gill (Plate 34) and at the same time, there was hyperemia (Plate 35) and deformation of the secondary lamella (Plate 35). The group treated with 150mg/L of the extracts showed severe destruction of the gills (Plate 36) enlarged primary lamellae and infilterations of polymorphonuclear cells, (Plate 37). Furthermore, deformed tips of the lamella and curling and deformation of the tips of the lamella (Plate 38 and 39) were observed in the groups treated with 250mg/L, 350mg/L and 450mg/L, respectively at the end of the 96h exposure. The groups treated with aqueous extracts of *M. oleifera* (D - G) had similar histopathological changes in their gill structure when compared to the gills of the normal control, positive control (standard drug), before treatment and group B (infected not treated).

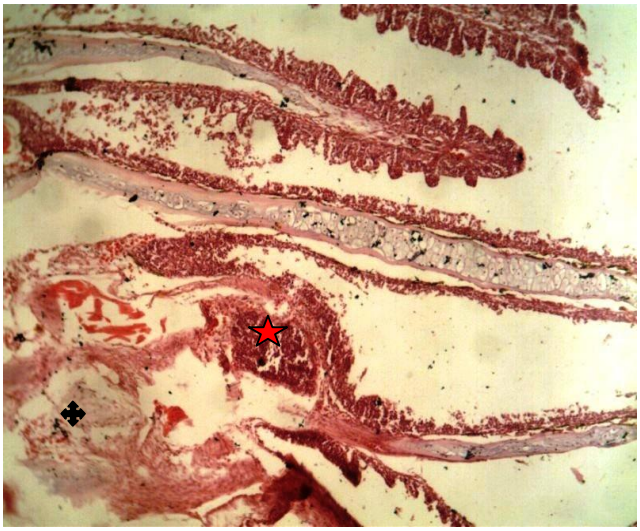

Plate 33: Infected gill of *Clarias gariepinus* before treatment (pre treatment) showed severe aneurysm (red star) and oedema (+) were evident. Deformation and complete fusion of the gill lamellae was discovered. H&E. mag 400X.

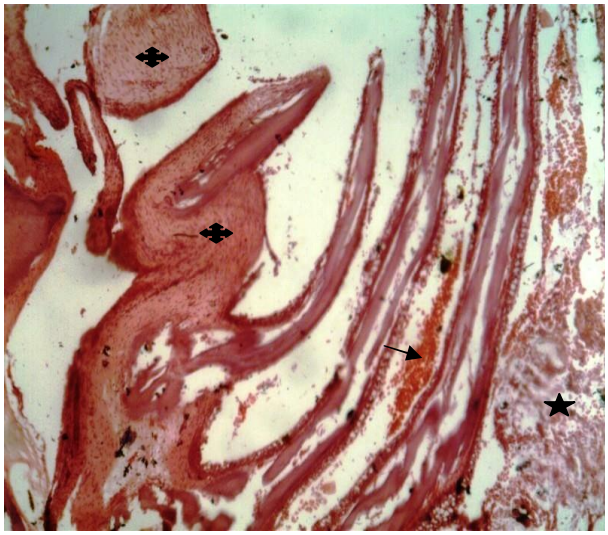

Plate 34: Photomicrograph of infected gill of *Clarias gariepinus* in group B (infected not treated) showed multiple epitheliocystis (+), congestion of red blood cells (black arrow) and severe oedema (star). H&E. mag 400X**.**

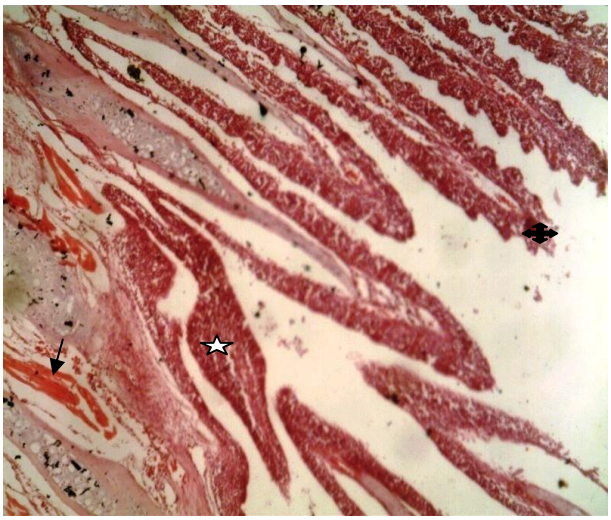

Plate 35: Photomicrograph of infected gill of *Clarias gariepinus* after 96h treatment with standard drug (fish cure) (Group C). There is fusion (+) and deformation of secondary lamella (*) and possible hyperemia/or engorgement (arrow). H&E. mag 400X.

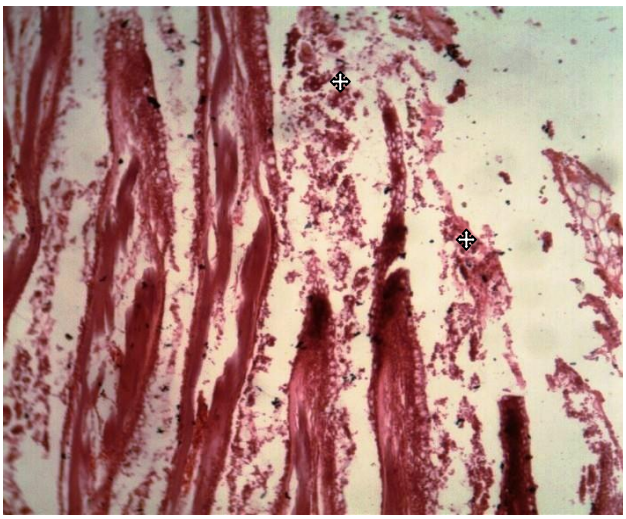

Plate 36: Photomicrograph of infected gill of *Clarias gariepinus* after 96h treatment with 150mg/L aqueous extracts of *Moringa oleifera* (Group D), showed severe destruction of the gill architecture showing complete loss of secondary lamella. H&E. mag. 400X.

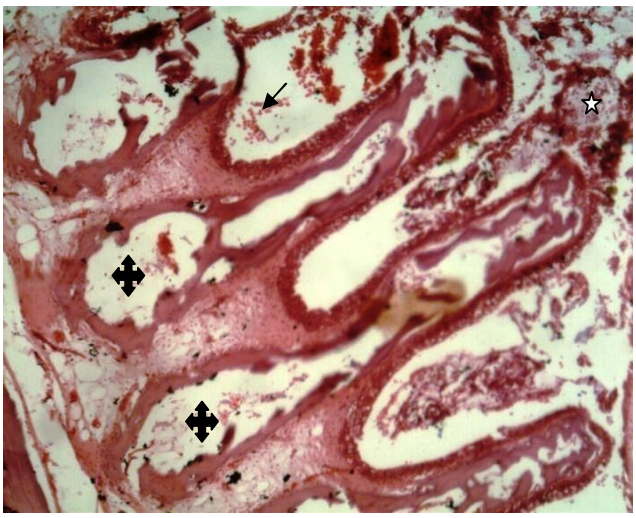

Plate 37: Photomicrograph of group E gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* after 96h treatment with 250mg/L aqueous extracts of *Moringa oleifera* showed dilatation or enlarged primary lamella (+), severe destruction of the tips of the secondary lamellae (*) and infilteration of polymorphonuclear cells (arrow). H&E. mag. 400X.

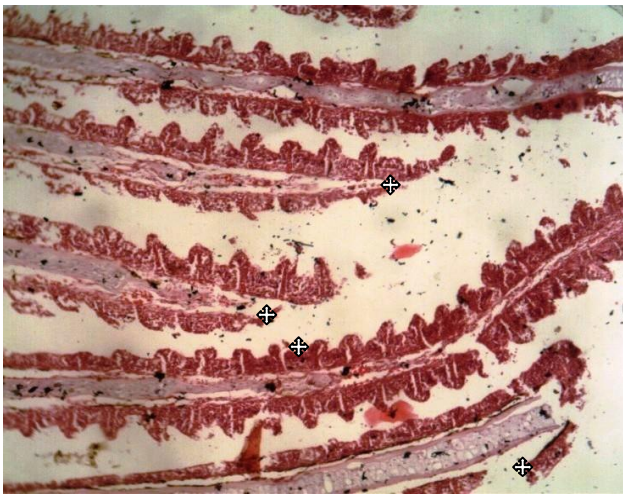

Plate 38: Photomicrograph of group F gill of *C. gariepinus* infected with *Ichthyophthirius multifilis* after 96h treatment with 350mg/L aqueous extracts of *Moringa oleifera* showed deformed secondary lamella especially at their tips (+). H&E mag. 400X

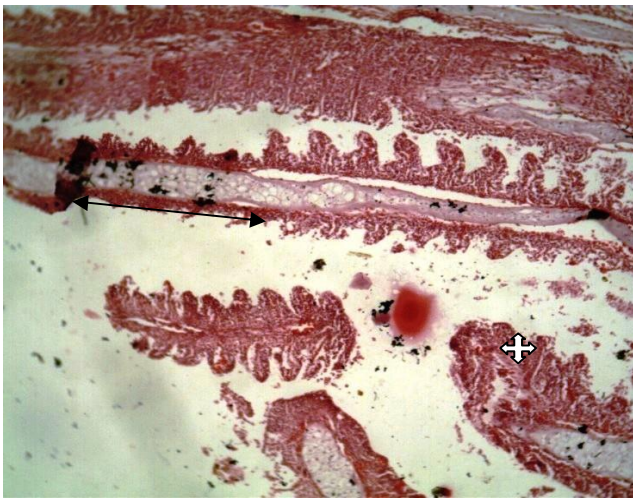

Plate 39: Photomicrograph of group F gill of *C. gariepinus* infected with *Ichthyophthirius multifilis* after 96h treatment with 450mg/L aqueous extracts of *Moringa oleifera* showed deformation of the secondary lamella (arrow head) and curling of the tips of lamella (+). H&E. mag 400X.

3.9.6. Histopathological changes in the gills of *Clarias gariepinus* exposed to prolonged bath treatments of aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

The gills section from the normal control was normal (Plate 28). Secondary lamellae of gill before treatment were completely fused and deformed (Plate 40) whereas some of the secondary lamellae of the gills of infected not treated (group B) were atrophied or dystrophied (Plate 41). The group C treated with standard drug showed that the gills were equally fused (Plate 42). However, the groups (D - G) treated with varied concentrations of *M. oleifera aqueous* extracts had hypertrophic/fused secondary lamellae of the gills, (Plate 43), eroded secondary lamellae (Plate 44) and enlarged primary lamellae and curling of the secondary lamellae with increased vascular congestion with infiltration of the submucosa by inflammatory cells (Plate 45 and Plate 46), respectively.

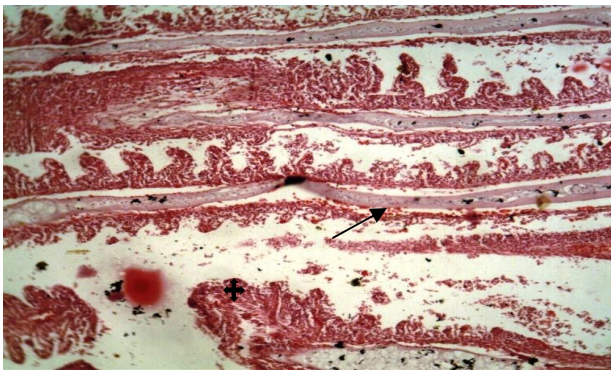

Plate 40: Gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* before treatment showed fusion (black cross) and deformed secondary lamellae (black arrow). H&E. mag. 400X.

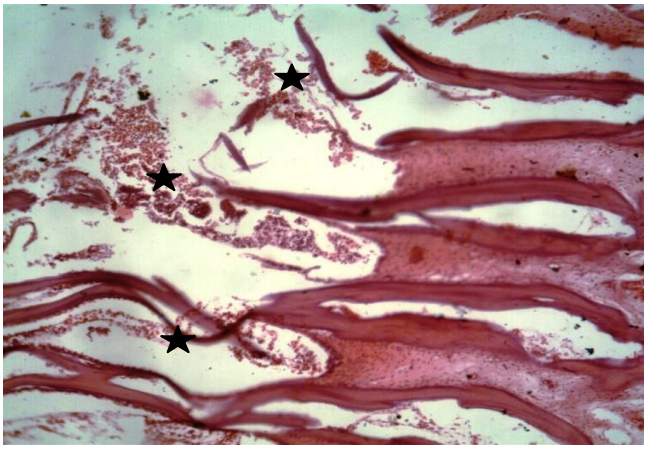

Plate 41: Gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* (group B-infected not treated) at the end of 15 days of exposure. The gill exhibited complete atrophy of the secondary gill lamella (star). H&E. mag. 400X.

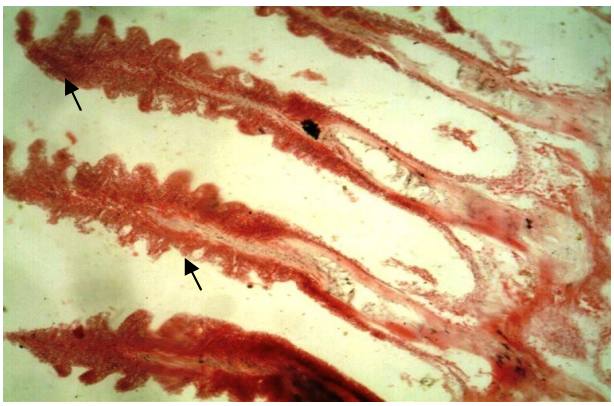

Plate 42: Photomicrograph of group C gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* treated with standard drug (fish cure) Fusion of secondary lamellae (black arrow).H&E. mag. 400X.

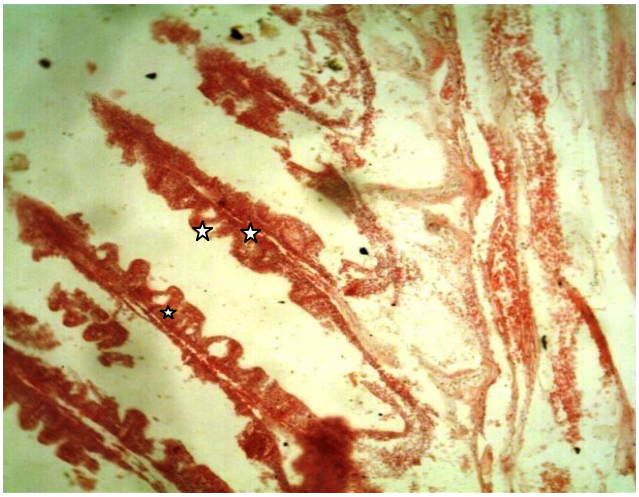

Plate 43: Photomicrograph of group D gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* treated with 15mg/L aqueous extracts of *Moringa oleifera* showing hypertrophy of the lamellae (star), fusion of the tip of secondary lamella (arrow). H&E. mag. 400X.

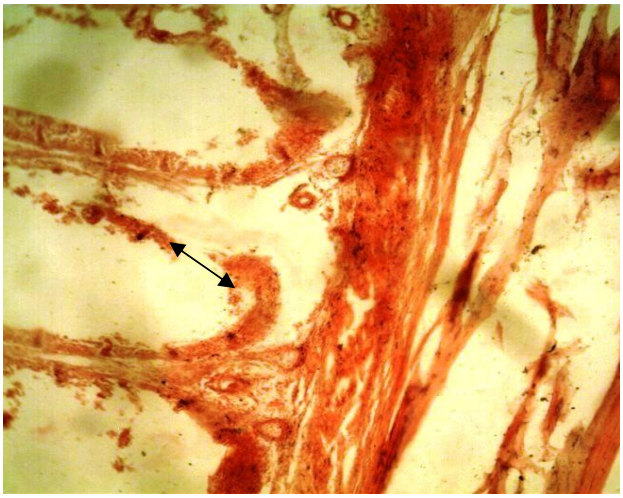

Plate 44: Photomicrograph of group E gill of *C. gariepinus* infected with *I. multifilis* treated with 25mg/L aqueous extracts of *Moringa oleifera*. Gill exhibited erosion of the secondary lamella (black arrow), enlarged primary lamella (star). H&E. mag. 400X.

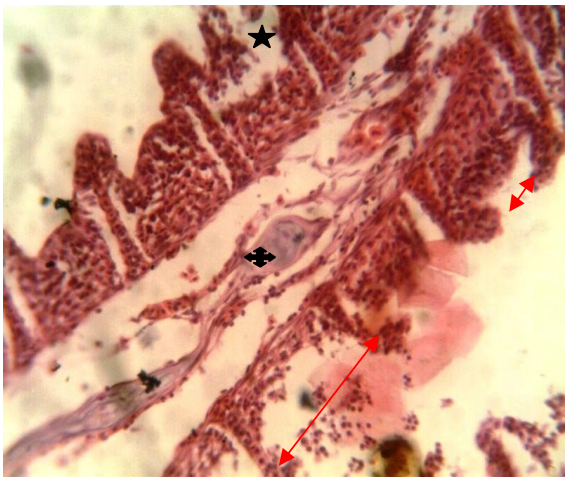

Plate 45: Photomicrograph of group F gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* treated with 35mg/L aqueous extracts of *Moringa oleifera*. There was destruction of the secondary lamella-curling (red arrow), epithelial lifting (*) oedema at the primary lamella (+) and proliferation of leukocytic infilterates (white arrow). H&E. mag. 400X.

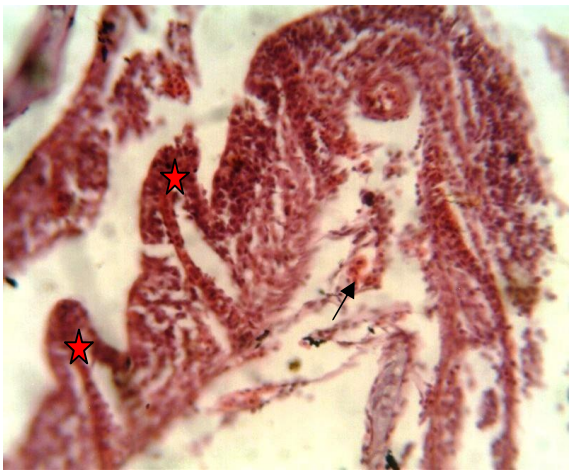

Plate 46: Photomicrograph of group G gill of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* treated with 45mg/L aqueous extracts of *Moringa oleifera*. Curling of the tips of the secondary lamella (*), erosion of the secondary lamella (+) and penetration of leukocytic infilterates (arrow). H&E. mag. 400X.

3.10. Proximate Composition of *Clarias gariepinus* Infested with *Ichthyophthirius multifilis* During Dip, Short Term and Prolonged Bath Treatments with Aqueous Extracts of *Moringa oleifera* and Standard Drug (Fish Cure)

The results of the proximate composition of the infested fish determined at the end of the dip treatments that lasted for 1h, are shown in Tables 11 and 20. Results showed that the protein levels before dip treatments did not differ significantly but differed significantly ($P<0.05$) at the end of the treatment period that lasted for 1h. The fat content of the infested fish showed no significant difference ($P<0.05$) before and after treatments. Similarly, the carbohydrate levels before treatment showed no significant difference ($P>0.05$), whereas, significant differences at ($P>0.05$) were recorded in their carbohydrate levels at the end of the treatment period that lasted for 1h. The moisture contents showed no significant difference ($P<0.05$) before and after dip treatments.

Table 19: Changes in the proximate composition of *Clarias gariepinus* Infested with *Ichthyophthirius multifilis* before dip treatments (1h) with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Protein	Fat	Carbohydrate	Moisture
A	14.66±1.69^{ab}	2.20±0.18^{bc}	3.88±2.25^{ab}	77.08±0.76^{a}
B	14.02±1.05^{a}	1.934±0.22^{a}	1.67±0.13^{a}	79.60±0.85^{a}
C	13.59±0.63^{a}	2.46±0.09^{c}	8.64±8.05^{b}	77.52±0.00^{a}
D	15.97±0.39^{b}	2.36±0.13^{bc}	1.52±1.27^{a}	77.95±1.42^{a}
E	13.49±0.39^{a}	2.12±0.11^{a}	3.32±2.83^{ab}	78.08±2.49^{a}
F	14.33±0.39^{a}	2.29±0.11^{bc}	1.84±0.78^{a}	79.10±0.52^{a}
G	14.57±0.31^{ab}	2.32±0.09^{bc}	0.97±0.17^{a}	79.65±0.52^{a}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). A (control), B (infected not treated), C (infected treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

Table 20: Change in proximate compositions of *Clarias gariepinus* after dip treatments with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Protein	Fat	Carbohydrate	Moisture
A	15.27±1.81^{b}	2.39±0.07^{b}	2.31±2.19^{a}	77.62±0.47^{b}
B	14.02±0.13^{ab}	2.25±0.05^{ab}	3.53±0.91^{a}	77.91±0.89^{ab}
C	13.46±0.39^{ab}	2.02±0.43^{a}	3.13±0.57^{a}	79.03±0.84^{b}
D	13.10±0.84^{ab}	2.18±0.25^{ab}	5.07±3.31^{ab}	77.44±3.63^{b}
E	11.92±1.65^{a}	2.20±0.32^{ab}	4.93±1.76^{ab}	78.66±0.55^{b}
F	14.13±1.60^{ab}	1.96±0.06^{a}	7.22±1.08^{b}	74.30±0.83^{a}
G	11.74±1.55^{a}	2.43±0.04^{b}	5.52±1.73^{ab}	77.97±1.72^{b}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). A (control), B (infected not treated), C (infected treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

In Table 21, it was observed that the protein level of the Ich infested fish showed no significant difference ($P>0.05$) before the treatment among the experimental groups. Likewise, after the short-term treatment (0 - 96h) there was no significant change ($P>0.05$) in the protein levels of the infested fish among the treated groups. The fat content in the infested fish did not differ significantly among the experimental group (A-G) ($P>0.05$) before and after treatment. The fat levels were all similar throughout the 96h exposure period.

Moreso, no significant difference ($P>0.05$) was observed in the carbohydrate levels of all the Ich infested fish at the beginning of the treatment but their carbohydrate level differed significantly ($P<0.05$) at the end of dip treatments that lasted for 1h. Their moisture contents did not differ significantly ($P>0.05$) in the treated groups before and after treatment. Moreover, the nutritional composition of all infested fish which includes; the protein, fats, carbohydrate and moisture did not show a wide variation in their contents when compared to the various concentrations of the extracts and the standard drug used in the treatment of the Ich infested fish The rationale for the proximate determination is because of the fact that Ich parasite is an ectoparasite that had the potential of causing skin lesion which will lead to other possible opportunistic infections that may possibly affect the nutrients levels. After short term bath treatments, the carbohydrate contents in most Ich infested fish in some treated group was found to be as low as 2.93±1.09% and high as 6.81±2.39% in group E and group C, respectively before treatment. In contrast, the group C was found to have carbohydrate level before treatment as low as 3.56±0.60% and high as 10.48±3.30 after treatment in group C and group E, respectively. At the end of the 15 days exposure, there was a significant difference ($P<0.05$) among the treated groups. The moisture content in general, during the prolonged bath treatments showed that the moisture content was high in group B and E and lower in groups C, D , F and G even though

they did not differ significantly ($P>0.05$) among the groups before treatment. Similarly, no significant difference ($P>0.05$) was observed in the moisture content of the infested fish at the end of the treatment period.

Table 21: Changes in the proximate composition of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* before and after short-term treatments with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Protein	Fat	Carbohydrate	Moisture
	Before treatment			
A (Healthy control)	15.03±0.97[a]	2.17±0.4[a]	3.77±0.60[a]	76.58±0.49[b]
B (infested not treated)	13.35±0.81[a]	2.53±0.10[ab]	4.73±1.43[a]	76.91±0.71[b]
C (standard drug)	13.53±1.48[a]	2.42±0.11[ab]	5.11±3.05[a]	76.59±1.62[b]
D (150mg/L)	12.09±2.47[a]	2.41±0.17[ab]	10.42±1.50[b]	72.72±1.64[a]
E (250mg/L)	13.29±2.22[a]	2.57±0.23[ab]	6.46±3.54[ab]	75.32±1.28[ab]
F (350mg/L)	13.27±2.15[a]	2.33±0.007[ab]	6.24±4.31[ab]	75.92±2.61[b]
G (450mg/L)	12.19±1.02[a]	2.93±0.84[b]	8.10±2.59[ab]	74.66±1.10[ab]
	After treatment			
Experimental group	Protein	Fat	Carbohydrate	Moisture
A (Healthy control)	14.58±0.64[a]	2.24±0.15[ab]	4.87±1.77[a]	75.94±2.13[a]
B (infested not treated)	12.44±2.19[a]	1.744±0.63[a]	2.31±0.17[b]	72.83±2.18[a]
C (standard drug)	13.67±0.86[a]	2.92±0.49[b]	2.49±0.36[ab]	75.18±0.44[a]
D (150mg/l)	13.44±1.41[a]	2.34±0.05[ab]	236±0.17[a]	77.01±3.65[a]
E (250mg/L)	12.40±1.15[a]	2.59±0.311[b]	2.37±0.32[ab]	73.27±2.65[a]
F (350mg/L)	13.71±0.87[a]	2.53±0.24[b]	2.37±0.31[ab]	72.74±1.69[a]
G (450mg/L)	13.62±1.22[a]	2.29±0.25[ab]	2.58±0.14[ab]	73.35±1.09[a]

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05).

From Table 22, it was observed that protein levels in the infested fish showed significant difference ($P>0.05$) before and after prolonged bath treatments. The fats level in the experimental group did not differ significantly at $P>0.05$ but was significantly significant at $P<0.05$ after prolonged treatment. Before treatment, the carbohydrate contents in most Ich infested fish in some treated groups was found to be as low as 2.93±1.09% and high as 6.81±2.39 % in group E and Group C, respectively. In contrast, the group C was found to have carbohydrates level before treatment as low as 3.56±0.60% and high as 10.48±3.30% after treatment in group C and E, respectively. At the end of the 15 days exposure, there were significant differences ($P<0.05$) in the protein and carbohydrates levels among the treated groups. Moreso, the moisture content in general, during the prolonged bath treatment was found to be higher in group B and E and lower in group C, D, F and G although, no significant difference ($P>0.05$) was observed among them. Similarly, no significance difference ($P>0.05$) as observed in the moisture content of the infested fish at the end of the treatment period Table 23.

Table 22: Changes in the proximate composition of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* before and after prolonged bath treatments with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Protein	Fat	Carbohydrate	Moisture
A (Healthy control)	16.65 ± 1.45^{c}	2.22 ± 0.10^{a}	1.35 ± 0.51^{a}	77.61 ± 1.44^{a}
B (infested not treated)	13.61 ± 0.53^{b}	2.04 ± 0.09^{a}	3.24 ± 1.48^{ab}	78.97 ± 1.37^{a}
C (standard drug)	12.77 ± 1.45^{ab}	2.33 ± 0.20^{a}	6.81 ± 2.39^{b}	75.86 ± 0.74^{a}
D (1,500mg/l)	11.53 ± 1.48^{a}	2.44 ± 0.33^{a}	6.62 ± 2.84^{b}	77.31 ± 1.00^{a}
E (2,500mg/L)	$14.03\pm0.0.76^{a}$	2.35 ± 0.10^{a}	2.93 ± 1.09^{ab}	78.42 ± 1.56^{a}
F (3,500mg/L)	13.22 ± 0.17^{ab}	2.31 ± 0.56^{a}	5.72 ± 1.93^{ab}	76.41 ± 1.75^{a}
G (4,500mg/L)	13.29 ± 0.59^{ab}	2.15 ± 0.08^{a}	5.98 ± 4.08^{b}	76.35 ± 3.53^{a}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$).

Table 23: Changes in proximate composition of *Clarias gariepinus* after prolonged bath treatment (15 days) with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Protein	Fat	Carbohydrate	Moisture
A (Healthy control)	16.45±0.70^{c}	2.26±0.23^{a}	2.12±0.76^{a}	76.78±1.18^{ab}
B (infested not treated)	15.20±4.59^{bc}	2.13±0.04^{a}	9.47±3.57^{b}	73.754.96^{a}
C (standard drug)	12.11±0.98^{ab}	2.41±0.18^{a}	3.56±0.60^{a}	79.55±0.64^{b}
D (15mg/l)	10.85±1.52^{a}	2.38±0.11^{a}	8.76±2.61^{b}	75.37±3.89^{ab}
E (25mg/L)	12.28±0.95^{ab}	2/34±0.32^{a}	10.48±3.30^{b}	72.23±2.67^{a}
F (35mg/L)	11.68±0.64^{ab}	2.53±0.24^{a}	9.11±2.16^{b}	74.33±2.51^{ab}
G (45mg/L)	13.14±2.10^{abc}	2.42±0.31^{a}	7.85±2.11^{b}	74.11±0.39^{a}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05).

3.11. Changes in the Packed Cell Volume (%) (PCV) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* Treated with Aqueous Extractss of *M. oleifera* after Dip, Short Term and Prolonged Bath Treatment.

The effect of the increasing concentrations of *M. oleifera* after dip treatments produced a duration dependent significant increase ($P<0.05$) in the PCV levels of infested fish. Before and after dip treatments (Ih), significant differences ($P< 0.05$) were observed among the treated groups. PCV levels were highest in the standard drug treated group with 57.85% increase when compared to other treated groups. The infested fish in group D has a markedly reduced PCV when treated with 1500mg/L of *M. oleifera* by 31.57%, at 2,500mg/L of *M. oleifera*, the PCV level increased by 39.46% followed by 3,500mg/L *M. oleifera* treated group which increased the PCV levels of the infested fish by 42.06%. Whereas at 4,500mg/L it was increased by 28.89% after the duration of the treatment when compared to the negative control at the end of Ih exposure with reduced PCV of 41.53% (Table 24).

During the short-term treatment, the effect of the reduced, concentrations (150mg/L, 250mg/L, 350mg/L and 450mg/L) in the PCV levels showed a duration dependent significant ($P< 0.05$) increase in the beginning, 72h and 96h exposure, compared to the normal control. On the other hand, no significant change ($P>0.05$) were observed in the PCV levels of the infested fish at 24h and 48h exposure period and among the treated groups. At the same time, the PCV levels of the infested fish when compared to the negative control at the end of 24h showed that at 150mg/L, the PCV level was increased by 7% followed by 19.31% increase in 250mg/L *M. oleifera* and finally by 15.7% increase at 450mg/L after the duration of treatment while negative

control had 28.05% reduction in PCV and 47.36% reduction in PCV by the positive control (Table 25).

However, during the prolonged bath treatments, the effect of the selected concentrations f (15mg/L, 25mg/L, 35mg/L and 45mg/L) produced a duration dependent significant difference ($P<0.05$) in the PCV levels of the infested fish at the end of Day 7 and Day 15 exposures. However, at the beginning of the experiment, no significant changes ($P>0.05$) were observed among the treated groups. Both the standard drug and the 45mg/l *M. oleifera* extracts had increased PCV levels in the infested fish by 62.5%, followed by 15mg/L and 25mg/L *M. oleifera* extractss with both increasing the PCV level by 50%, whereas, at 35mg/L the PCV had 37.5% increase after the duration of treatment compared with the negative control at Day 7 which had a 0% change (Table 26).

Table 24: Changes in the PCV (%) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* before and after dip treatments with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	After treatment (1h)	Percentage change
A	20.0 ± 1.10^{c1}	21.67 ± 2.52^{c1}	
B	13.0 ± 1.0^{a1}	12.67 ± 2.51^{a1}	41.53
C	15.67 ± 1.53^{a1}	2.10 ± 1.0^{bc2}	57.85
D	15.33 ± 0.58^{a1}	18.67 ± 1.15^{bc1}	-31.57
E	I6.53±1.53^{b1}	17.67 ± 0.53^{b1}	39.46
F	16.33 ± 2.52^{b1}	18.0 ± 2.0^{bc1}	42.06
G	14.10 ± 2.6^{ab1}	16.33 ± 3.06^{b1}	28.89

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different numbers in a row are signinficantly different (P<0.05). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

Table 25: Changes in the PCV (%) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* before and after short term treatment with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	24h	48h	72h	96h	% change
A	21.10±2.65^{b1}	20.67±1.15^{abc1}	20.67±2.52 ^{ab1}	23.33±1.15^{bc1}	23.33±1.53^{b1}	-
B	16.10±1.0^{a1}	19.00±2.0^{a1}	17.33±4.93^{a1}	15.67±1.53^{a1}	13.67±1.53^{a1}	-28.05
C	15.33± 0.58^{a1}	23.0±1.0^{c1}	24.67±4.16^{b1}	25.33±0.58^{c1}	28.0±1.0^{c1}	-47.36
D	15.67±2.08^{a1}	22.3±2.08^{bc1}	25.0±1.0^{b1}	20.67±0.58^{b1}	20.33±1.53^{b1}	7
E	15.33±1.53^{a1}	19.67±1.53^{a1}	22.33±2.08^{ab1}	21.67±2.52^{b1}	22.67±1.53^{b1}	19.31
F	15.33±0.58^{a1}	22.33±2.08^{bc1}	23.67±1.53^{b1}	20.67±2.52^{b1}	24.33±0.58^{bc1}	28.05
G	17.33±3.06^{a1}	19.67±1.53^{a1}	23.0±2.65^{b1}	21.33±1.53^{b1}	22.0±5.57^{b1}	15.7

Mean values with different numbers in a row are signinficantly different (P<0.05).Mean values with different alphabets as superscript in a column are significantly different (P<0.05). A (control), B (infected not treated), C (infected treated with standard drug), D(150mg/L), E(250mg/L), F(350mg/L) and G (450mg/L).

Table 26: Changes in the PCV (%) of *Clarias gariepinus* infested with *Ichthyophthirus multifilis* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Treatment	Before treatment	Day 7	Day 15	% change
A	19.10 ± 1.0^{a1}	20.67 ± 2.52^{b1}	20.67 ± 2.52^{c1}	-
B	17.67 ± 3.06^{a1}	8.0 ± 1.0^{a2}	8.0 ± 1.0^{a2}	-
C	18.33 ± 4.04^{a1}	15.0 ± 5.19^{a1}	13.0 ± 5.2^{b1}	62.5
D	21.33 ± 2.31^{a1}	12.0 ± 2.0^{a2}	12.0 ± 2.0^{a2}	50
E	17.67 ± 0.58^{a1}	12.0 ± 1.0^{a1}	12.0 ± 1.0^{b1}	50
F	18.0 ± 3.46^{a1}	11.0 ± 2.65^{a1}	11.0 ± 2.65^{ab1}	37.5
G	18.33 ± 1.53^{a1}	13.0 ± 2.0^{a1}	13.0 ± 2.0^{ab1}	62.5

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

3.12 Changes in the Red Blood Cell Count (x10^6/L) of *Clarias gariepinus* Infested with *Ichthyophthirius multifilis* Treated with Aqueous Extracts of *Moringa oleifera* after Dip, Short Term and Prolonged Bath Treatments with Aqueous Extracts of *Moringa oleifera* Leaves and Standard Drug (Fish Cure).

The effect of the increasing, concentrations of *M. oleifera* (1500mg/L, 2,500mg/L, 3,500mg/l and 4,500mg/L) after dip treatments produced a duration dependent significant difference ($P<0.05$) at the end of Ih exposure. In addition no significant different ($P>0.05$) was recorded in RBCs concentrations before treatment among the treated groups. In the 1,500mg/L treated groups, the RBCs were increased by 13.62%.

Moreover, at 2,500mg/L concentration, it was increased by 15.5%, at 3500mg/L, the RBCs level was increased by 13.79% while at 4,500mg/L, it only increased the RBCs by 5.28%. The RBCs level in the group treated with standard drug increased by 12.26%. whereas the negative control at the end of Ih exposure, had a 27.97% RBCs reduction (Table 27).

Moreover, the Ich infested fish exposed to short term treatments (0 - 96h) had a significantly increased RBCs ($P<0.05$) before treatments at, 72h and 96h exposures. The extractss (150mg/L, 250mg/L, 350mg/L and 450mg/L) produced a concentration dependent significant increase in the RBCs of the infested fish. At the end of the 96h exposure when compared to the negative control at 24h exposure results, showed that 150mg/L increased RBCs by 40.62%, 250mg/L increased RBCs by 33.38%, and 350mg/L increased it by 29.68% while the 450mg/l increased it by 31.93%. The negative control had a 3.5% increase in the RBCs when compared to other treated groups (Table 28).

The varied concentrations (15mg/L, 25mg/L, 35mg/L and 45mg/L) of *M. oleifera* extracts produced significant increases ($P<0.05$) in RBCs levels at the end of Day 15 exposure but the increases were not concentration dependent throughout the exposure period. In 15mg/L treated groups, the RBCs decreased by 1.82%, whereas in 25mg/L, 35mg/L and 45mg/L, the RBCs levels of the infested fish increased by 8.02%, 27.37% and 1.83% respectively when compared to the negative control in Day 7 with RBCs reduced by 4.37% (Table 29).

Table 27: Changes in RBCs (x10^{12}/L) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* during dip treatment with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	After treatment	% change
A	7.44 ± 0.37^{b1}	8.15 ± 0.40^{b1}	
B	5.74 ± 0.31^{a1}	5.87 ± 0.59^{a1}	-27.97
C	6.17 ± 0.12^{ab1}	6.59 ± 0.26^{a1}	12.26
D	6.58 ± 0.54^{ab1}	6.67 ± 0.53^{a1}	13.62
E	6.65 ± 0.66^{ab1}	6.78 ± 0.78^{a1}	15.5
F	6.01 ± 0.64^{a1}	6.68 ± 0.63^{a1}	13.79
G	6.17 ± 0.88^{ab1}	6.18 ± 0.39^{a1}	5.28

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

Table 28: Changes in RBCs (x10^{12}/L) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* during short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Exp. Group	Before	24h	48h	72h	96h	% change
A	7.68 ± 0.31^{b1}	7.05 ± 0.81^{a1}	7.62 ± 1.07^{ab}	7.05 ± 0.71^{a1}	7.33 ± 0.43^{b1}	-
B	6.14 ± 0.31^{a1}	6.67 ± 1.25^{a1}	7.42 ± 0.44^{a1}	8.34 ± 1.09^{b1}	6.91 ± 0.53^{b1}	3.5
C	$6.58\pm0.24^{a\,1}$	7.27 ± 0.86^{a1}	7.92 ± 0.69^{ab1}	8.77 ± 0.23^{b1}	8.76 ± 0.76^{b1}	31.33
D	6.16 ± 0.81^{a1}	7.18 ± 0.22^{a1}	8.65 ± 0.26^{b1}	8.67 ± 0.27^{b1}	9.38 ± 0.71^{b1}	40.62
E	6.16 ± 0.81^{a1}	7.18 ± 0.22^{a1}	8.65 ± 0.26^{b1}	8.67 ± 0.27^{b1}	8.89 ± 0.74^{b1}	33.28
F	6.75 ± 0.29^{a1}	7.54 ± 0.32^{a1}	8.35 ± 0.22^{ab1}	8.76 ± 0.30^{b1}	8.65 ± 0.83^{b1}	29.68
G	6.41 ± 0.03^{a1}	7.06 ± 0.57^{a1}	8.29 ± 0.36^{ab1}	8.02 ± 0.47^{ab1}	8.82 ± 0.47^{b1}	31.93

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$). (Control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

Table 29: Changes in the RBCs(x10^{12}/L) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Treatment groups	Before treatment	Day 7	Day 15	% change
A	6.63 ± 0.61^{a1}	6.11 ± 0.79^{a1}	6.64 ± 0.68^{cd1}	-
B	5.47 ± 0.71^{a1}	5.48 ± 1.89^{a1}	5.24 ± 1.30^{a1}	-4.37
C	5.98 ± 0.50^{a1}	5.98 ± 0.24^{a1}	6.48 ± 0.49^{bcd1}	18.25
D	6.05 ± 0.21^{a1}	6.87 ± 2.14^{a1}	5.38 ± 0.38^{ab1}	-1.82
E	6.38 ± 0.87^{a1}	7.37 ± 1.53^{a1}	5.92 ± 0.41^{abcd1}	8.02
F	6.04 ± 1.16^{a1}	6.15 ± 0.83^{a1}	6.98 ± 0.12^{d1}	27.37
G	5.45 ± 0.72^{a1}	6.69 ± 0.46^{a1}	5.58 ± 0.24^{abc1}	1.83

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$). (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E(25mg/L), F(35mg/L) and G (45mg/L).

3.13 Changes in the Haemoglobin (Hb) (g/dl) Levels of *Clarias gariepinus* Infested with *Ichthyophthirius multifilis* Treated with Aqueous Extracts of *Moringa oleifera* Leaves and Standard Drug (Fish Cure) after Dip, Short term and Prolonged bath Treatments

Among the varied concentrations of the extractss studied after Ih duration of treatment, *M. oleifera* at 3,500mg/L and 4500mg/L showed that Hb increased by 10.09% and 10.09% respectively in comparison with 1,500mg/L and 2,500mg/L, which had 6.8% and 2.1% increase in Hb levels in the infested fish. In the negative control, the haemoglobin level reduced drastically by 14.08% whereas the positive control (standard drug) group, had increased Hb of 2.1% (Table 30).

In addition, the haemoglobin levels in the Ich infested fish during short term treatments, differed significantly throughout the exposure period ($P<0.05$). The significant increase in the HB levels among the treated groups was duration dependent. The haemoglobin level in the standard drug treated groups increased by 19.95% after the duration of the treatments. The haemoglobin levels among the varied concentration of the extractss treated groups at 150mg/L, 250mg/l, 350mg/L, and 450mg/L increased by 24.49%, 22.75%, 19.54% and 32.5%, respectively, after the duration of the treatment compared with the negative control at 24h (Table 31).

The haemoglobin levels throughout the prolonged bath treatment exposure period (Day 7 and Day 15) were statistically significant ($P<0.05$) whereas no significant difference ($P>0.05$) was observed before treatments. At the same time, among the extracts treated groups, the Hb levels increased by 25.23%, 25.81%, 29.03% and 26.56% in 15mg/L, 25mg/L, 25mg/L, 35mg/L and 45mg/L concentrations, respectively. However, the Hb level increased by 44.97% in the Ich-

infested fish treated with standard drug compared to the negative control that had 11.38% reduced Hb level (Table 32).

Table 30: Changes in haemoglobin levels (g/dl) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* and treated with aqueous extracts of *M. oleifera* leaves and standard drug (fish cure).

Exp. Group	Before treatment	After treatment	% change
A	9.17 ± 0.45^{c1}	9.23 ± 0.42^{c1}	-
B	7.33 ± 0.29^{ab1}	7.93 ± 0.58^{a1}	-14.08
C	7.77 ± 0.32^{b1}	8.10 ± 0.1^{ab1}	2.1
D	7.80 ± 0.36^{b1}	8.10 ± 0.46^{ab1}	6.8
E	7.57 ± 0.57^{ab1}	8.10 ± 0.10^{ab1}	2.1
F	7.47 ± 0.55^{b1}	8.73 ± 0.12^{bc1}	10.09
G	6.80 ± 0.36^{a1}	8.77 ± 0.49^{bc1}	10.59

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

Table 31: Changes in the haemoglobin levels (g/dl) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Exp. Group	Before Treatment	24h	48h	72h	96h	% change
A	8.60 ± 0.53^{a1}	8.87 ± 60.23^{bc1}	9.27 ± 0.45^{b1}	9.10 ± 0.70^{b1}	8.77 ± 10.15^{a1}	-
B	6.67 ± 1.15^{a1}	7.47 ± 0.47^{a1}	7.73 ± 0.42^{a1}	7.90 ± 0.44^{a1}	7.65 ± 0.81^{a1}	2.14
C	8.00 ± 0.56^{a1}	8.20 ± 0.70^{ab1}	8.33 ± 0.23^{b1}	9.50 ± 0.35^{b1}	8.96 ± 0.70^{ab1}	19.95
D	7.83 ± 0.11^{a1}	8.70 ± 0.65^{bc1}	8.67 ± 0.12^{ab1}	9.17 ± 0.23^{b1}	$8.96\pm0.70^{ab\ 1}$	24.49
E	7.73 ± 1.09^{a1}	8.93 ± 09.64^{b1c}	9.10 ± 0.17^{ab1}	9.57 ± 0.23^{b1}	9.17 ± 1.22^{ab1}	22.75
F	7.30 ± 1.73^{a1}	9.03 ± 0.25^{bc1}	8.80 ± 0.46^{ab1}	9.47 ± 0.40^{b1}	8.93 ± 1.36^{ab1}	19.54
G	8.30 ± 1.04^{a1}	9.43 ± 0.38^{c1}	9.10 ± 0.20^{b1}	9.10 ± 0.30^{b11}	9.9 ± 1.40^{b1}	32.5

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

Table 32: Changes in haemoglobin (g/dl) levels of *Clarias gariepinus* infested with *Ichthyophthirus multifilis* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Exp. group	Before treatment	Day 7	Day 15	% Change
A	7.37 ± 0.46^{a1}	7.96 ± 0.51^{a1}	6.60 ± 0.7^{b1}	-
B	7.13 ± 1.0^{a1}	5.27 ± 0.21^{b1}	4.67 ± 1.07^{a1}	-11.38
C	7.33 ± 1.08^{a1}	5.90 ± 0.7^{b1}	7.64 ± 1.34^{b1}	44.97
D	6.63 ± 1.29^{a1}	6.13 ± 0.25^{b1}	6.60 ± 0.7^{b1}	25.23
E	7.27 ± 1.14^{a1}	5.77 ± 0.64^{b1}	6.63 ± 0.58^{b1}	25.81
F	6.57 ± 0.46^{a1}	5.50 ± 0.36^{b1}	6.8 ± 1.22^{b1}	29.03
G	6.43 ± 0.59^{a1}	5.40 ± 0.46^{b1}	6.67 ± 0.64^{b1}	26.56

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

3.14 Changes in the White Blood Cell Count (WBC) (x10^9/L) of *Clarias gariepinus* Infested with *Ichthophthirus multifilis* Treated with Dip, Short term and Prolonged bath Treatments with Aqueous Extracts of *Moringa oleifera* Leaves and Standard Drug (fish cure)

During the dip treatments, significant differences ($P< 0.05$) were observed in the WBC of infested fish before and after treatments. Comparing extracts treated groups, WBCs levels were increased by 9.67%, in 1500mg/L *M. oleifera* while increased WBCs of 4.15% was recorded in 4,500mg/L treated groups. On the other hand, the WBCs levels were reduced by 0.46% in both 2,500mg/L and 3500mg/L treated groups. WBCs had a significant reduction by 30.89% in the negative control after the 1h duration treatment (Table 33).

White blood cell counts in the infested fish showed significant differences ($P<0.05$) among the treated groups at the end of 24h and 96h exposure. However, no significant differences ($P>0.05$) was recorded in 48h and 72h exposures. White blood cells level were slightly increased by 3.50% at 150mg/L followed by 9.39% increase at 250mg/L,14.39% increase at 250mg/L, 14.39% increase at 350mg/L and 5.05% increase at 450mg/L. Similarly, WBC levels increased in the standard drug treated group by 3.50% compared to the negative control with 12.45% WBC levels increase at 24h (Table 34).

No significant differences ($P<0.05$) were recorded in WBC levels throughout the exposure period. Among the extracts treated groups, the WBC levels increased by 22.85% at 15mg/L, followed by reduced WBCs of 11.91% at 350mg/L, and reduced WBCs of 19.57% at 25mg/L while at 45mg/L, the WBCs reduced drastically by 24.25% (Table 35).

Table 33: Changes in the WBCs(x10^9/L) level in *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental Group	Before Treatment	After treatment	% change
A	11300.00±2051.83^{b1}	10466.67±1159.02^{b1}	-
B	7200.00±900.0^{a1}	7233.33±208.17^{a1}	-30.89
C	6700.12±264.58^{a1}	7366.67±896.27^{a1}	1.84
D	7166.67±1006.64^{a1}	7933.33±757.19^{a1}	9.67
E	7300.00±100.00^{a1}	7200.11±1153.26^{a1}	-0.46
F	6666.67±416.33^{a1}	7200.00±888.82^{a1}	-0.46
G	6500.0±529.15^{a1}	7533.33±776.75^{a1}	4.15

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different numbers in a row are signinficantly different (P<0.05). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

Table 34: Changes in the WBCs(x10^9/L) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after short term treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Exp. Group	Before Treatment	24h	48h	72h	96h	% change
A	7666.67±838.65^{a1}	8233.33±665. 83^{a1}	8266.67±776.75^{a1}	8100.00±700.0^{a1}	7966.67±76.75^{a1}	-
B	7033.33±635.09^{a1}	7566.66±305.51^{a1}	8933.33±152.75^{a1}	9300.10±100.0^{b2}	9633.33±208.17^{bc2}	12.45
C	7266.67±472.52^{a1}	8033.33±152.75^{a1}	8633.33±851.19^{a1}	8700.10±655.74^{ab1}	8.866.67±550.76^{b1}	3.50
D	7266.67±1096.97^{a1}	8300.0±793.73^{a1}	8566.67±288.67^{a1}	8866.67±642.9^{ab2}	8866.67±208.17^{b1}	3.50
E	7700.10±721.11^{a1}	8633.33±152.75^{a2}	9066.67±416.33^{a1,2}	9333.33±404.15^{b2}	8410.00±556.78^{bc1}	9.39
F	6733.33±100.0^{a1}	8433.33±1001.67^{a2}	8633.33±152.75^{a2}	8910.0±608.28^{ab1}	9800.0±100.0^{c2}	14.39
G	7300.10±100.0^{a1}	8433.33±1001.69^{a1}	8800.10±458.26^{a1}	9066.67±35119^{ab1}	9000.00±173.2^{bc2}	5.05

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different numbers in a row are signinficantly different (P<0.05). A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

Table 35: Changes in the WBCs(x10^9/L) level in *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental groups	Before treatment	Day 7	Day 15	Percentage change
A	7066.67±808.29^{a1}	6166.67±57.74^{a1}	5900.00±624.49^{a2}	-
B	7400.0±1135.78^{a1}	7833.33±550.78^{b1}	7866.67±550.76^{ab1}	0.42
C	6700.0±608.27^{a1}	5833.3±404.15^{a1}	6500.0±608.28^{ab1}	-17.02
D	6933.33±550.76^{a1}	6400.0±624.49^{ab1}	7166.67±950.44^{ab1}	22.85
E	6866.67±493.29^{a1}	6233.33±1150.36^{a1}	6300.0±1374.77^{ab1}	-19.57
F	6733.33±288.68^{a1}	5833.33±602.77^{a1}	6900.0±1374.77^{ab1}	-11.91
G	7266.67±907.38^{a1}	6766.67±1514.38^{ab1}	5933.33±472.58^{a2}	-24.25

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different numbers in a row are signinficantly different (P<0.05). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

3.15 Changes in the Differential Counts (Lymphocytes and Neutrophils) of *Clarias gariepinus* Infested with *Ichthyophthirius multifilis* Treated with Dip, Short term and Prolonged bath Treatments with Aqueous Extracts of *Moringa oleifera* Leaves and Standard Drug (fish cure).

In the infested fish, the lymphocyte counts among the treated groups during dip treatments showed significant differences ($P<0.05$) at the end of the 1h treatment but did not differ significantly ($P>0.05$) before treatment. Lymphocyte counts were significantly higher in the normal control groups at the end of 1h exposure compared to all other treatment groups whereas it was significantly higher in all groups at the same period. The lymphocyte counts were reduced by 0.45% at 1500mg/L extracts, while the lymphocyte count reduced by 5.67% and 0.86% but increased by 3.07% at 4,500mg/L, 3,500mg/L and 2,500mg/L, *M. oleifera* extracts, respectively after the duration of the treatment compared with negative control at 1h (Table 36)

The variation in the lymphocyte counts among the treated groups before treatments and at the end of 96h exposure showed significant differences ($P<0.05$) but did not differ significantly ($P>0.05$) at 24h, 48h and 72h exposures. Among the *M. oleifera* treated groups, lymphocyte counts increased by 29.9% at 150mg/L and 30.97% at 450mg/L. The positive control (standard drug) group showed an increased lymphocyte count by 26.08% compared to the negative control with increased lymphocyte count of 27.18% at 24h (Table 37).

The lymphocyte counts in the Ich-infested fish did not differ significantly ($P>0.05$) among the treated groups before treatment and Day 7 exposure. At the end of the prolonged exposure period (Day 15) significant differences ($P<0.05$) were recorded among the treated groups. *M. oleifera* either increased or reduced lymphocyte counts in a concentration

independent manner across the duration of the study with 15mg/L extracts increasing lymphocyte counts by 4.9% while at 25mg/L, 35mg/L and 45mg/L, they were reduced by 6.69%, increased by 1.78% and increased by 1.34%, respectively in comparison with negative control at Day 7 having increased lymphocyte count of 10.71% (Table 38).

The Neutrophils count before and after treatment was not statistically significant ($P<0.005$) among the treated groups. However, the neutrophil count was reduced by 10.13% at 1500mg/L, and 2,500mg/L at 3,500mg/L it was reduced by 7.26%, at 4,500mg/l it was reduced drastically 18.83%. The neutrophil counts in the group treated with standard drug were reduced by 7.26% compared with negative control at 1h (Table 39).

The beginning (before treatment) and end of the short term (96h) exposure period showed significant decrease ($P<0.05$) in the neutrophil counts among the treated groups. The decrease in neutrophil count was duration dependent and concentration independent. *M. oleifera* at 150mg/L increased neutrophil counts by 0.51%, at 250mg/L but was reduced by 38.9% at 350mg/L it was increased by 8.4% while at the highest concentration of the extracts (450mg/L) the neutrophil counts were reduced by 5.08%. The neutrophil counts in the Ich-infested fish treated with standard drug showed 1.72% reduction in the neutrophil count compared to the negative control with increased neutrophil counts by 5.08% at 1h (Table 40).

The neutrophils counts before treatment and Day 7 showed significant differences ($P<0.05$) though they did not differ significantly ($P>0.05$) at the end of the prolonged bath treatments (15days). However, among the *M. oleifera* treated groups, the neutrophil counts increased by 54.09% at 15mg/L, 32.44% at 25 mg/L, 35.19% at 35mg/l and 37.88% at 45mg/L (Table 41).

Table 36: Changes in the lymphocyte counts of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Exp. Group	Before treatment	After treatment	% change
A	86.00 ± 1.0^{a1}	25.00 ± 2.0^{c2}	-
B	78.33 ± 9.24^{ab1}	76.35 ± 3.52^{ab1}	10.2
C	85.67 ± 4.93^{b1}	71.33 ± 3.21^{a1}	-6.5
D	79.33 ± 1.15^{ab1}	76.67 ± 3.06^{ab1}	0.45
E	76.00 ± 5.29^{ab1}	78.67 ± 4.16^{b1}	3.07
F	56.67 ± 36.17^{a1}	75.67 ± 2.89^{ab1}	-0.86
G	78.67 ± 6.11^{ab1}	72.00 ± 4.10^{a1}	-5.67

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

Table 37: Changes in the lymphocyte counts of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Exp. Group	Before treatment	24h	48h	72h	96h	% change
A	78.67 ± 1.15^{ab1}	80.67 ± 7.02^{a1}	77.67 ± 2.52^{ab1}	84.0 ± 5.29^{a}	78.0 ± 2.64^{a1}	
B	76.33 ± 5.68^{ab1}	61.33 ± 27.30^{a1}	80.67 ± 1.15^{b1}	79.33 ± 5.13^{a1}	78.0 ± 3.46^{a1}	27.18
C	70.0 ± 2.0^{a1}	73.33 ± 3.05^{a1}	73.67 ± 5.51^{a1}	79.67 ± 0.58^{a1}	77.33 ± 3.05^{a1}	26.08
D	73.33 ± 3.05^{ab1}	71.33 ± 1.53^{a1}	78.0 ± 3.46^{ab1}	78.0 ± 3.46^{a1}	79.67 ± 1.53^{a1}	29.9
E	82.00 ± 2.00^{b1}	74.33 ± 6.02^{a1}	75.67 ± 4.93^{ab1}	76.0 ± 3.46^{a1}	87.0 ± 6.08^{b1}	41.86
F	72.67 ± 4.62^{ab1}	78.33 ± 10.4^{a1}	77.33 ± 2.52^{ab1}	77.0 ± 9.64^{a1}	78.35 ± 2.08^{a1}	27.72
G	80.00 ± 6.72^{b1}	77.67 ± 2.52^{a1}	81.0 ± 1.0^{b1}	75.00 ± 4.36^{a1}	80.33 ± 0.58^{a1}	30.97

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

Table 38: Changes in the lymphocyte counts of *Clarias gariepinus* infested with *Ichthyophthirus multifilis* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Exp. Group	Before treatment	Day 7	Day 15	% Change
A	77.67 ± 5.13^{a1}	76.33 ± 3.21^{ab1}	710 ± 1.0^{ab1}	
B	79.33 ± 4.04^{a1}	74.67 ± 4.73^{ab1}	82.67 ± 1.53^{c1}	10.71
C	76.67 ± 4.16^{a1}	79.0 ± 4.36^{b1}	79.0 ± 2.65^{bc1}	-5.79
D	70.0 ± 2.0^{a1}	72.0 ± 8.19^{ab1}	78.33 ± 4.04^{bc1}	4.9
E	79.33 ± 5.77^{a1}	72.0 ± 1.73^{ab1}	69.67 ± 2.31^{a1}	-6.69
F	73.33 ± 5.77^{a1}	71.67 ± 10.06^{ab1}	76.0 ± 5.00^{abc1}	1.78
G	69.67 ± 7.64^{a1}	65.0 ± 4.0^{a1}	75.67 ± 8.39^{abc1}	1.34

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

Table 39: Changes in the neutrophil counts of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Exp. Group	Before treatment	After treatment	% change
A	16.67 ± 4.93^{ab1}	18.10 ± 4.58^{a1}	
B	22.67 ± 2.31^{b1}	23.00 ± 4.58^{a1}	27.78
C	13.00 ± 4.36^{a1}	24.67 ± 5.03^{a2}	7.26
D	19.33 ± 1.15^{ab1}	20.67 ± 3.06^{a1}	-10.13
E	22.10 ± 7.21^{b1}	20.67 ± 3.06^{a1}	-10.13
F	18.33 ± 1.15^{ab1}	21.33 ± 2.31^{a1}	-7.26
G	19.10 ± 1.10^{ab1}	18.67 ± 4.73^{a1}	-18.83

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different numbers in a row are signinficantly different (P<0.05).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

Table 40: Changes in the Neutrophil counts of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Exp. Group	Before Treatment	24h	48h	72h	96h	%change
A	19.33 ± 1.15^{a1}	14.67 ± 8.08^{a1}	21.33 ± 3.05^{a1}	14.67 ± 6.11^{a1}	21.33 ± 2.31^{b1}	-
B	23.00 ± 6.08^{ab1}	19.67 ± 1.53^{a1}	18.0 ± 2.0^{a1}	17.00 ± 7.21^{a1}	20.67 ± 3.06^{b1}	5.08
C	27.33 ± 3.06^{b1}	26.33 ± 3.21^{a1}	25.0 ± 7.0^{a1}	19.10 ± 1.10^{a1}	19.33 ± 1.15^{a1}	-1.72
D	26.33 ± 3.21^{b1}	29.33 ± 0.58^{a1}	21.0 ± 3.61^{a1}	20.67 ± 3.00^{a1}	19.79 ± 0.58^{b1}	0.51
E	16.67 ± 1.15^{a1}	25.33 ± 5.03^{a2}	24.10 ± 5.19^{a2}	16.33 ± 3.21^{a1}	12.10 ± 5.29^{a1}	-38.9
F	26.33 ± 4.73^{b1}	27.33 ± 3.06^{a1}	22.00 ± 2.00^{a1}	18.67 ± 8.08^{a1}	21.33 ± 2.30^{b1}	8.4
G	19.33 ± 1.15^{a1}	19.33 ± 2.05^{a1}	17.33 ± 1.15^{a1}	20.33 ± 4.51^{a1}	18.67 ± 1.15^{b1}	-5.08

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

Table 41: Changes in the neutrophils counts of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Exp. Group	Before treatment	Day 7	Day 15	% Change
A	17.00 ± 2.10^{a1}	18.10 ± 1.10^{b1}	13.67 ± 1.15^{a1}	
B	18.67 ± 2.08^{a1}	12.33 ± 2.08^{a1}	20.33 ± 3.06^{b1}	64.88
C	18.67 ± 1.53^{a1}	19.10 ± 1.10^{b1}	16.33 ± 2.52^{ab1}	32.44
D	20.3 ± 1.53^{a1}	17.67 ± 3.05^{b1}	$19,10\pm2.0^{b1}$	54.09
E	20.0 ± 1.0^{a1}	19.33 ± 0.58^{b1}	16.33 ± 2.31^{ab1}	32.44
F	18.0 ± 1.0^{a1}	15.33 ± 1.53^{ab1}	$16.67\pm\pm0.58^{ab1}$	35.19
G	24.10 ± 4.36^{a1}	17.10 ± 4.10^{b1}	17.00 ± 3.61^{ab1}	37.88

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

3.16. Changes in the Blood Electrolytes of *Clarias gariepinus* Infested with *Ichthyophthirius multifilis* and Treated with Aqueous Extracts of *Moringa oleifera* Leaves and Standard Drug (Fish Cure) after Dip, Short term and Prolonged bath Treatments

3.16.1. Changes in the sodium levels (mEq/L) of *Clarias gariepius* infested with *Ichthyophthirius multifilis* and treated with aqueous extracts of *M. oleifera* leaves and standard drug (fish cure) after dip treatments

The sodium levels in the serum of the infested fish showed that the standard drug treated group had the highest level of sodium with values from 121.24 ± 4.21mEq/L to 115.13 ± 18.1mEq/L though the increase did not differ significantly ($p > 0.05$). At the same time, the groups treated with varied concentrations of *M. oleifera* showed significant decrease in sodium levels from 118.68 ± 2.10 to 80.85 ± 28.61mEq/L, 110.59 ± 8.39mEq/L to 101.32 ± 1.34mEq/L, 113.84 ± 5.1mq/L to 98.13 ± 43.41mEq/L and 117.37 ± 11.79mEq/L to 99.86 ± 33.18mEq/L, for groups D, E, F and G before and after treatments, respectively,. However, the infected but not treated group differed significantly before and after treatments when compared to the normal control that maintained similar mean values. However, significant differences ($p < 0.05$) were observed among the treated groups before treatment but no significant differences were recorded at the end of the 1h dip treatments among the experimental groups. (Table 42).

Table 42. Changes in the sodium levels (mEq/L) of *Clarias gariepinus* exposed to *Ichthyophthirius multifilis* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before Treatment	After Treatment (1h)
A	81.88 ± 25.38^{a1}	82.55 ± 39.54^{a1}
B	104.207 ± 13.78^{b1}	112.94 ± 10.161^{a}
C	121.24 ± 4.21^{b1}	115.13 ± 18.1^{a1}
D	118.68 ± 2.10^{b1}	80.85 ± 28.61^{a2}
E	110.59 ± 8.39^{b1}	101.32 ± 11.34^{a1}
F	113.84 ± 5.15^{b1}	98.13 ± 43.41^{a1}
G	117.37 ± 11.79^{b1}	99.86 ± 35.18^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers in a row are signinficantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E(2500mg/L), F(3500mg/L) and G (4500mg/L).

3.16.2. Changes in the sodium levels (mEq/L) of *Clarias geriepnus* infested with *Ichthyophthirius multifilis* and treated with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) after short term treatments

The mean daily sodium levels of Ich infested *C. gariepinus* treated with varied concentrations of *M. oleifera* and standard drug are shown in Table 43. Sodium levels were highest in 48h in group D treated with 250mg/L *M. oleifera* extracts, but declined rapidly thereafter. Treatment D had the highest sodium level though not significantly different ($p > 0.05$) when compared to other groups. Sodium levels were lowest in the negative control in 24h when compared to other treated groups and normal control but increased progressively during the exposure period. The increases were significantly different ($p < 0.05$). Moreover, before treatment, the sodium levels among the treatment groups were statistically significant ($p<0.05$) but did not differ significantly ($p > 0.05$) among the treated groups from 24-96h exposures.

Table 43: Changes in the sodium levels (mEq/L) of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	24h	48h	72h	96h
A	93.26 ± 18.23^{a1}	101.01 ± 14.84^{a1}	90.15 ± 17.64^{a1}	112.37 ± 59.6^{a1}	79.34 ± 20.49^{a1}
B	125.13 ± 4.48^{b1}	89.68 ± 14.03^{a2}	114.82 ± 61.02^{a1}	$139.69 \pm 35.0^{a3,2}$	164.99 ± 39.3^{2b4}
C	133.85 ± 25.4^{b1}	121.26 ± 33.85^{a1}	132.98 ± 52.68^{a1}	118.27 ± 11.3^{a1}	127.30 ± 35.88^{ab1}
D	132.64 ± 9.36^{b1}	106.43 ± 25.13^{a2}	156.70 ± 27.88^{a3}	127.91 ± 5.02^{a1}	122.33 ± 51.29^{ab1}
E	121.98 ± 2.37^{b1}	117.68 ± 13.13^{a1}	125.08 ± 16.7^{a1}	125.42 ± 16.1^{a1}	129.85 ± 13.22^{ab1}
F	123.41 ± 6.55^{b1}	118.36 ± 21.08^{a1}	$133.25 \pm 10.51^{a1,2}$	116.35 ± 6.66^{a1}	140.44 ± 32.43^{ab2}
G	129.69 ± 1.57^{b}	129.46 ± 7.89^{a1}	134.21 ± 15.77^{a1}	134.92 ± 36.7^{a1}	134.01 ± 24.88^{ab1}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05).
Mean values with the different numbers as superscript in a row are not significantly different (P>0.05).
A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

3.16.3 Changes in the sodium levels (mEq/L) of *Clarias gariepinus* infested with ich and treated with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) after prolonged bath treatments.

The mean weekly sodium levels of ich infested *C. gariepinus* treated with varied concentration of *M. oleifera* and standard drugs are shown in Table 44. Sodium levels were highest in day 7 in group E treated with 350mg/L of the extracts, and declined significantly thereafter. The highest sodium levels observed in group E were significantly different ($p<0.05$) in day 7. Whereas,the lowest sodium levels were reorded in group D at the end of day 7 but increased rapidly at the end of the treatment period. Similar trend of sodium levels increase was observed in group B treated groups, with significant increase in sodium levels throughout the duration of exposure. Meanwhile, sodium levels declined in group E, F and G and standard control (C) from day 7 and day 15. The decline were significantly different ($p < 0.05$) and duration dependent. Comparing the mean values from the column, no significant difference ($p > 0.05$) were recorded among the treatment groups before treatment and at the end of the treatment periods. Though the sodium levels in the normal control (group A) were lower throughout the exposure periods, they were not statistically significant ($p < 0.05$).

Table 44: Changes in the sodium levels (mEq/L) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before Treatment	Day 7	Day 15
A	91.71 ± 12.64^{a1}	108.96 ± 11.63^{a1}	103.70 ± 10.63^{a1}
B	114.89 ± 13.52^{b1}	116.50 ± 23.43^{b1}	140.84 ± 35.41^{b2}
C	133.95 ± 51.09^{ab1}	133.24 ± 6.48^{a1}	114.62 ± 12.58^{ab1}
D	119.40 ± 12.52^{ab1}	106.28 ± 6.85^{a1}	126.58 ± 9.34^{ab2}
E	123.17 ± 9.51^{ab1}	130.40 ± 14.85^{a1}	115.00 ± 13.07^{ab1}
F	111.05 ± 12.01^{ab1}	125.63 ± 13.84^{a1}	117.72 ± 9.115^{ab1}
G	123.47 ± 6.34^{ab1}	120.26 ± 26.68^{a}	116.95 ± 5.95^{ab1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with the different alphabets as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D(15mg/L), E(25mg/L), F(35mg/L) and G (45mg/L).

3.16.4. Changes in the potassium levels (mEq/L) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* and treated with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) after dip treatments.

The potassium levels of ich infested *C. gariepinus* treated with varied concentrations of *M. oleifera* and standard drugs are shown in Table 45. Potassium levels were highest in the group B (Negative control) and increased rapidly thereafter. The increase were statistically significant ($p < 0.05$), when compared to other groups. Low levels of potassium were recorded in the normal control and the standard drug treated groups, but the decline was not significantly different ($p > 0.05$). However, before treatment, the potassium levels among the treated groups and the controls did not differ significantly ($p > 0.05$), whereas significant differences were recorded among the mean potassium levels of the experimental groups ($p < 0.05$) at the end of the 1h dip treatment. Moreover, among the extracts treated groups, potassium levels increased at the end of the 1h treatment exposure when compared to their baseline. But the increased potassium levels observed among the extracts treated groups were not higher when compared to the negative control group.

Table 45: Change in the potassium levels (mEq/L) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	After Treatment
A	6.32 ± 2.85^{a1}	6.01 ± 2.31^{a1}
B	8.16 ± 1.44^{a1}	14.29 ± 3.86^{c1}
C	7.18 ± 2.66^{a1}	6.93 ± 1.98^{a1}
D	6.18 ± 1.01^{a1}	8.29 ± 0.86^{ab1}
E	7.82 ± 1.59^{a1}	7.79 ± 2.03^{a1}
F	7.55 ± 0.81^{a1}	13.11 ± 1.68^{bc1}
G	7.14 ± 1.99^{a1}	10.38 ± 2.80^{abc1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$ Mean values with the different alphabets as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

3.16.5. Changes in the potassium levels (mEq/L) of *Clarias gariepinus* infested with *Ichthyophthirius multifilis* and treated with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) after short term treatments

The potassium levels of *C. gariepinus* infested with ich and treated with aqueous extracts of potassium levels were highest in the group C at the end of 48h of exposure but declined rapidly thereafter (Table 46). Treatment C has the highest potassium level and differed significantly ($p < 0.05$) when compared to the baseline and normal control. Moreover, the lowest potassium levels were recorded in group G treated with 450mg/L of the extracts at the end of 72h treatment. However, mixed trends of potassium levels were recorded among the treated groups and controls. At the same time, among the experimental groups before treatment, no significant differences ($p > 0.05$) were recorded in the mean potassium levels of the infested fish. Like wise at the end of the 24h and 96h exposures, the potassium levels of the treated groups, negative and normal control did not differ significantly ($p > 0.05$). But in 48h and 72h treatment periods, potassium levels among the treated groups and the controls (negative and normal) differed significantly ($p < 0.05$).

Table 46: Changes in the potassium levels (mEq/L) **of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).**

Experimental group	Before Treatment	24h	48h	72h	96h
A	4.61 ± 0.34^{a1}	3.36 ± 1.42^{a1}	5.65 ± 3.43^{a1}	3.88 ± 0.76^{ab1}	5.34 ± 0.62^{a1}
B	5.20 ± 0.45^{a1}	7.66 ± 3.00^{b1}	4.82 ± 3.77^{a1}	6.15 ± 0.98^{c1}	2.84 ± 2.11^{a1}
C	5.22 ± 0.28^{a1}	3.64 ± 1.86^{a1}	12.81 ± 1.15^{b2}	3.73 ± 1.15^{ab1}	3.50 ± 1.57^{a1}
D	5.17 ± 0.41^{a1}	4.67 ± 1.53^{ab1}	12.69 ± 1.5^{a1}	5.35 ± 0.29^{bc1}	3.83 ± 2.31^{a1}
E	$4.56 \pm 1\text{-}11^{a1}$	4.01 ± 1.51^{a1}	10.27 ± 0.96^{b2}	3.53 ± 1.60^{ab1}	3.91 ± 1.21^{a1}
F	6.85 ± 3.35^{a1}	3.07 ± 1.59^{a1}	11.25 ± 0.85^{b2}	4.00 ± 0.76^{ab1}	4.27 ± 1.82^{a1}
G	6.91 ± 2.09^{a1}	3.3 ± 2.05^{a1}	11.79 ± 6.69^{b2}	2.74 ± 1.18^{a1}	4.41 ± 1.05^{a}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with the different alphabets as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F(350mg/L) and G (450mg/L).

3.16.6. Changes in the potassium levels (mEq/L) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 47 shows that mean weekly potassium levels of ich infested C. *gariepinus* treated with varied concentration of *M. oleifera* were statistically significant ($p < 0.05$) in day 7. The potassium level in group D declined a little at the end of the prolonged bath treatments. The lowest potassium level was observed in the normal control with a mean value of 3.28 ± 111mEq/L and the decline did not differ significantly during the exposure period ($p > 0.05$). However, mixed trends were seen in the potassium levels of the treated groups and the controls (negative and normal) when compared to their baselines values. Moreover, before treatment while comparing the mean value along the column, no significant differences ($p < 0.05$) were observed among the groups. However, at the end of day 7 and day 15, there were significant differences ($P<0.05$) in potassium levels.

Table 47: Changes in the potassium levels (mEq/L) **of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).**

Experimental group	Before treatment	Day 7	Day 15
A	6.50 ± 1.66^{ab1}	3.28 ± 1.11^{a2}	5.21 ± 0.44^{a1}
B	7.59 ± 1.54^{ab1}	5.34 ± 1.03^{a1}	6.52 ± 1.48^{a1}
C	5.02 ± 0.13^{a1}	11.38 ± 3.02^{b2}	10.09 ± 1.66^{ab1}
D	8.21 ± 1.10^{b1}	19.22 ± 0.95^{c2}	15.67 ± 2.53^{bc2}
E	6.38 ± 2.22^{ab1}	16.31 ± 2.60^{bc2}	17.38 ± 4.97^{c2}
F	7.86 ± 2.46^{ab1}	$14.74 \pm 4.0b^{c2}$	15.62 ± 4.11^{bc2}
G	8.49 ± 1.10^{b1}	13.12 ± 5.26^{b1}	15.22 ± 3.62^{bc1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with the different alphabets as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D(15mg/L), E(25mg/L), F(35mg/L) and G (45mg/L).

3.16.7. Changes in the chloride levels (mEq/L) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 48 shows that mean chloride levels of ich infested *C.gariepnus* treated with varied concentrations of *M. oleifera* after 1h treatment was highest in group E. The increase was statistically significant ($p < 0.05$) when compared to the normal control. Moreso, the infected not treated (group B) had the lowest chloride levels when compared to the treated groups. Compared to the baselines, the chloride levels of the treated groups had a progressive marked increase in the chloride levels across the duration of exposure and the increase was statistically significant ($p < 0.05$) but the increase was not concentration dependent. Similar increases were observed between the normal and negative control. But the normal control did not differ significantly ($p > 0.05$) in chloride levels when compared with the negative control. Considering means along the column, the chloride levels were statistically significant ($p < 0.05$) before and after 1h treatments among the treated groups and the negative control when compared to the normal control.

Table 48: Changes in the chloride level (mEq/L) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental Group	Before Treatment	After Treatment (1h)
A	59.02 ± 8.51^{a1}	66.82 ± 36.29^{a1}
B	86.48 ± 8.80^{b1}	97.14 ± 23.75^{ab1}
C	92.47 ± 10.57^{b1}	108.32 ± 20.16^{b1}
D	96.75 ± 10.58^{b1}	$120.50 \pm 5.39^{b\,2}$
E	92.01 ± 11.49^{b1}	131.24 ± 21.17^{b2}
F	100.76 ± 8.13^{b1}	119.17 ± 4.96^{b1}
G	84.22 ± 15.57^{b}	116.94 ± 11.89^{b2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different alphabets as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

3.16.8. Changes in the chloride levels (mEq/L) of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

The mean daily chloride levels of ich infested *C. gariepinus* treated with varied concentrations of *M. oleifera* and standard drug are shown in Table 49. Chloride levels were higher in the group treated with 350mg/L of extracts. The chloride levels were significantly reduced in the standard drug treated group ($p < 0.05$) when compared with their baseline values (before treatments) in 24h and 72h, respectively. When compared with the chloride levels of each treated groups baselines, only group C treated group maintained a steady trend of the decline in chloride levels starting from 48h to 96h when compared to other treated groups that had a mixed trends in their chloride levels.

However, no significant differences ($p > 0.05$) where observed among the groups before the treatments while considering mean values in the column, and at 24h and 96h only. Furthermore, at the end of 48h and 72h treatment exposures, the mean chloride levels among the groups differed significantly ($p < 0.05$). Throughout the exposure period, the chloride levels in the normal control were stable when compared to other treated groups and negative control.

Table 49: Changes in the chloride levels (mEq/L) of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before Treatment	24h	48h	72h	96h
A	100.10 ± 20.35^{a1}	94.62 ± 8.88^{a1}	92.19 ± 17.45^{a1}	92.44 ± 15.88^{abc1}	88.22 ± 28.71^{a1}
B	117.56 ± 18.78^{a1}	$128.10 \pm 8.93^{a1,2}$	135.54 ± 10.3^{c2}	122.81 ± 11.56^{cd1}	128.03 ± 31.03^{ab1}
C	125.96 ± 9.32^{a1}	136.37 ± 46.28^{a1}	100.70 ± 18.75^{ab2}	87.81 ± 13.05^{ab3}	96.80 ± 21.12^{ab3}
D	120.70 ± 19.71^{a1}	117.96 ± 10.34^{a1}	11.19 ± 5.17^{bc1}	84.08 ± 27.95^{a2}	102.30 ± 20.39^{ab1}
E	133.39 ± 5.79^{a1}	119.44 ± 17.98^{a2}	125.29 ± 13.92^{c1}	116.47 ± 9.37^{bcd2}	131.99 ± 11.7^{b1}
F	117.01 ± 17.96^{a1}	132.81 ± 18.81^{a2}	$122.24 \pm 7.05^{bc1,2}$	127.57 ± 5.50^{d2}	123.67 ± 6.40^{ab2}
G	121.99 ± 21.99^{a1}	168.70 ± 9.43^{a2}	123.12 ± 8.11^{bc1}	120.94 ± 21.45^{cd1}	119.81 ± 15.0^{ab1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with the different alphabets as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

3.16.9. Changes in the chloride levels (mEq/L) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 48 shows the mean weekly chloride levels of ich infested *C. gariepinus* which was highest in group E treated with 25mg/L of the extracts at the end of day 7. The increase was statistically significant ($p < 0.05$) when compared to the control. Moreso, the lowest levels of the chloride were recorded in the normal control at the end of day 15 exposure. Meanwhile, comparing means values along the column in day 7, the chloride levels among the groups differed significantly ($p <0.05$) and at the same time increased across the duration in comparison with there baselines and decreased in day 15, whereas, the negative control had a progressive increase throughout the exposure period. The chloride levels among the groups at the end of day 15 and baselines were not significantly different ($p > 0.05$).

Table 50: Changes in the chloride levels (mEq/L) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	Day 7	Day 15
A	55.31 ± 25.12^{a1}	94.27 ± 24.30^{a2}	86.76 ± 11.74^{a2}
B	98.48 ± 16.24^{ab1}	120.32 ± 9.83^{b2}	133.55 ± 16.51^{b2}
C	101.26 ± 19.0^{ab1}	111.27 ± 8.91^{ab1}	$109.59 \pm 11.59a^{b1}$
D	103.44 ± 16.62^{b1}	132.17 ± 8.59^{b}	114.19 ± 15.68^{ab1}
E	109.82 ± 14.69^{b1}	137.01 ± 7.48^{b2}	116.27 ± 13.85^{b1}
F	100.85 ± 15.97^{ab1}	122.36 ± 20.01^{b2}	110.83 ± 9.74^{ab1}
G	131.80 ± 48.54^{b1}	133.28 ± 5.35^{b1}	111.97 ± 21.10^{ab2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$ Mean values with the different alphabets as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

3.16.10 Changes in the bicarbonate levels (mEq/L) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

The bicarbonate levels of *C. gariepinus* infested with ich and treated with aqueous extracts of *M. oleifera* leaves is shown in Table 51. At the end of the 1h dip treatments, the bicarbonate level was highest in the group B and the increase is not statistically significant ($p > 0.05$) when compared with other groups. Moreso, among the treated groups, group D had the lowest bicarbonate levels while the normal control had the lowest bicarbonate levels when compared to the baselines values of the Ich infested groups. The bicarbonate levels declined before treatment and also at the end of the 1h treatment among the groups. The decrease was time dependent and concentration independent. However, the negative control recorded similar decrease but the decrease was lower compared to the other groups that had higher decreases in bicarbonate levels. Meanwhile, before treatment, the mean bicarbonate levels among the groups differed significantly when compared to the normal control. However, no significant differences ($p >0.05$) were observed among the groups at the end of 1h treatment.

Table 51: Changes in the bicarbonate levels (mEq/L) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	After Treatment (1h)
A	22.66 ± 2.08^{a1}	22.33 ± 4.93^{a1}
B	52.33 ± 4.04^{b1}	49.33 ± 16.77^{b1}
C	41.00 ± 16.09^{b1}	38.33 ± 850^{ab1}
D	43.00 ± 15.72^{b1}	33.00 ± 6.08^{ab1}
E	45.66 ± 4.04^{b1}	33.33 ± 6.11^{ab1}
F	50.33 ± 1.52^{b1}	36.67 ± 2.89^{ab1}
G	47.33 ± 12.01^{b1}	40.00 ± 8.66^{b1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

3.16.11 Changes in the bicarbonate levels (mEq/L) after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

The bicarbonate levels in ich infested *C. gariepinus* after short term treatment with varied concentrations of *M. oleifera* and standard drug are shown in Table 52. The highest bicarbonate level was observed in the group B at the end of 24h and the increase was statistically significant at $p < 0.05$ while the lowest bicarbonate level was observed in the group C at the end of the short term treatments and the decrease was significantly lower ($p < 0.05$) when compared to other groups. Comparing the extractss treated groups; there bicarbonate levels had mixed trends. At the end of the exposure periods, the bicarbonate levels were lower compared to the initial baselines values of each individual group treated with varied concentrations of the extractss. At the same time, the group C had decreased levels of bicarbonate across the duration of exposure. The decrease was time dependent and significant at $p < 0.05$. The normal control group maintained reduced levels of bicarbonate throughout the exposure period when compared with the treated groups.

However, there were a significant differences ($p < 0.05$) in bicarbonate levels of the infested fish among the groups before treatments at 24h and 96h only. At 48h and 72h, no significant changes ($p > 0.05$) was observed in the bicarbonate levels of the Ich infested fish among the groups.

Table 52: Changes in bicarbonate levels (mEq/L) of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental Group	Before Treatment	24h	48h	72h	96h
A	20.15 ± 1.08^{a1}	26.01 ± 3.51^{a1}	27.45 ± 5.69^{a1}	22.58 ± 5.99^{a1}	21.84 ± 4.55^{a1}
B	32.79 ± 2.5^{c1}	37.11 ± 6.34^{b1}	22.64 ± 7.01^{a1}	31.61 ± 1.45^{b1}	32.98 ± 6.43^{b1}
C	27.49 ± 2.82^{b1}	28.58 ± 5.77^{a1}	27.26 ± 5.32^{a1}	26.70 ± 4.68^{ab1}	21.78 ± 3.99^{a1}
D	31.17 ± 3.97^{bc1}	29.65 ± 2.19^{ab1}	27.53 ± 2.90^{a1}	26.75 ± 4.66^{ab1}	25.25 ± 3.43^{a1}
E	30.90 ± 2.05^{bc1}	30.02 ± 0.32^{ab1}	32.51 ± 2.59^{a1}	24.51 ± 1.08^{ab1}	25.01 ± 3.43^{a1}
F	30.46 ± 1.79^{bc1}	24.69 ± 4.89^{a1}	26.90 ± 6.73^{a1}	25.22 ± 2.41^{ab1}	27.93 ± 0.11^{ab1}
G	27.82 ± 3.18^{bc1}	31.24 ± 0.96^{ab1}	24.67 ± 4.52^{a1}	27.50 ± 5.48^{ab1}	23.67 ± 5.23^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$).). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infected not treated), C (infected treated with standard drug), D(150mg/L), E(250mg/L), F(350mg/L) and G (450mg/L).

3.16.12 Changes in the bicarbonate levels (mEq/L) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

The mean weekly bicarbonate levels in Ich infested *C. gariepinus* showed that group D had the highest value of bicarbonate in day 7 and lowest in the normal control (Table 53). The increase was statistically significant at $p < 0.05$. Comparing the mean values of groups treated with the extractss and the duration of exposure, group D maintained a sharp decline in bicarbonate levels from pretreatment to post treatment. When the means along the column were compared, there were significant differences ($p < 0.05$) in the bicarbonate levels of the treated groups compared to the normal baseline control and all through the treatment periods. (day 7 and day 15).

Table 53: Changes in the Bicarbonate levels (mEq/L) **of *Clarias gariepinus* after prolonged bath treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).**

Experimental group	Before Treatment	Day 7	Day 15
A	21.52 ± 1.54^{a1}	18.91 ± 2.00^{a1}	29.17 ± 7.71^{ab1}
B	43.94 ± 6.47^{b1}	29.91 ± 1.62^{b2}	35.43 ± 5.94^{bc1}
C	32.96 ± 5.67^{ab1}	30.89 ± 2.07^{bc1}	22.28 ± 2.99^{a1}
D	42.41 ± 8.61^{a1}	40.60 ± 1.11^{e1}	31.28 ± 1.98^{abc1}
E	41.20 ± 9.92^{b1}	36.70 ± 4.71^{cde1}	39.02 ± 0.52^{c1}
F	35.91 ± 10.69^{ab1}	32.58 ± 4.91^{bcd1}	37.02 ± 7.01^{bc1}
G	33.47 ± 6.65^{ab1}	37.66 ± 4.75^{de1}	31.93 ± 4.63^{bc1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$).). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

3.17. Changes in the Antioxidant Status of *Clarias gariepinus* Infested with *Ichthyophthirius multifilis* and Treated with Aqueous Extracts of *Moringa oleifera* Leaves and Standard Drug (Fish Cure) during Dip, Short term and Prolonged bath Treatments

3.17.1. Changes in the catalase activity of *C. gariepinus* infested with Ich and treated with aqueous extracts of *M. oleifera* leaves and standard drug (fish cure) during dip treatments

The catalase activity in the gill tissue of ich infested *C. gariepinus* treated with varied concentrations of *M. oleifera* showed elevated levels of the antioxidant catalase activity in group G treated with 4,500mg/L recorded 2.49 ± 1.74µmol of H_2O_2/min/mg protein whereas the least level was recorded in group E treated with 1,500mg/L of the extracts having a value of 1.36± 0.72 catalase activity. However, in group D and F, there catalase levels in the gills increased with mean values of 1.38 ± 1.61 and 1.60 ± 0.79 µmol of H_2O_2/min/mg protein, respectively at the end of the dip treatments. At the same time, catalase activity in the gill tissues of group G was lower when compared to the group A (normal control) at the end of the dip treatments with mean value of 2.73 ± 3.45. The standard drug treated group recorded elevated levels of catalase activity when compared with the negative control that had reduced level of catalase activity in comparison with other groups. The catalase activity in each group when compared to their baselines (before treatment) and the end of the dip treatments showed no significant differences at $p > 0.05$ and the increase were time-dependent. Comparing the groups along the column, no significant different was recorded ($p > 0.05$) among the catalase activities in the gills of each group throughout the end of the 1h treatment. The normal control had the highest level which was not significant ($p > 0.05$) when compared to the standard drug, extracts groups and the negative control.

Furthermore, the catalase activity in the muscle of ich infested *C. gariepinus* was highest in the group B at the end of the 1h treatment with mean value of 2.43 ± 1.80 µmol of H_2O_2/min/mg protein compared to the extracts treated groups and the standard drugs. Comparing the extractss treated groups, the group treated with 2,500mg/L had the highest level of catalase activity with mean value 1.79 ± 0.76 µmol of H_2O_2/min/mg protein followed by the group D treated with 1,500mg/L of the extracts which had the lowest catalase activity in the muscle with mean value of 1.00 ± 0.13 µmol of H_2O_2/min/mg protein. At the same time, comparing each groups with their baselines (before treatment), there were reduced catalase activities in some extracts treated groups. Thus, the catalase activity in the muscle of the ich infested fish in group D (1,500mg/L) decreased when compared with the mean values of itôs baseline from 2.14 ± 1.53 µmol of H_2O_2-/min/mg protein to 1.00 ± 0.13 µmol of H_2O_2/min/mg protein while the group G (4,500mg/L) was from 2.46 ± 0.96 µmol of H_2O_2/min/mg protein (before treatment) to 1.71 ± 1.33 µmol of H_2O_2/min/mg protein after treatment. Meanwhile, the standard drug treated group had the lowest value of the catalase activity in the muscle when compared to the extracts groups and the infested not treated (negative control). The catalase activities in the muscle of the infested fish among the groups did not differ significantly ($p > 0.05$) before and after treatments (Table 54).

Table 54: Changes in Catalase activity (µmol of H_2O decomposed/min/mg protein) in the gills and muscles of *Clarias gariepinus* infected with *Ichthyophthirius multifilis* and treated with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure) during dip treatments

Experimental group	Tissue	Before treatment	After treatment (1h)
A	Gill	1.44 ± 0.04^{a1}	2.73 ± 3.45^{a1}
	Muscle	1.58 ± 05^{a1}	0.62 ± 0.34^{a2}
B	Gill	0.21 ± 0.41^{a1}	1.18 ± 1.69^{a1}
	Muscle	2.19 ± 1.75^{a1}	2.43 ± 1.80^{a1}
C	Gill	1.07 ± 1.01^{a1}	1.28 ± 1.47^{a1}
	Muscle	1.46 ± 2.07^{a1}	0.95 ± 0.24^{a2}
D	Gill	0.95 ± 0.21^{a1}	1.38 ± 1.61^{a1}
	Muscle	2.14 ± 1.53^{a1}	1.00 ± 0.13^{a1}
E	Gill	1.07 ± 1.02^{a1}	1.36 ± 0.72^{a2}
	Muscle	1.55 ± 1.03^{a1}	1.79 ± 0.76^{a1}
F	Gill	1.59 ± 1.36^{a1}	1.60 ± 0.79^{a1}
	Muscle	3.32 ± 1.10^{a1}	1.43 ± 0.48^{a2}
G	Gill	1.44 ± 1.04^{a1}	2.49 ± 1.74^{a1}
	Muscle	2.46 ± 0.96^{a1}	1.71 ± 1.33^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$).). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

3.17.2 Changes in the catalase activity in the gills and muscles of *C. gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

The catalase activity in the gills of the ich infested *C. gariepinus* among the extracts treated group (D-G) at the end of short term treatments (96h) was highest in group G but the increase was not significant ($p > 0.05$) whereas the lowest activity was recorded in the group D, though not significant ($p > 0.05$) when compared to the normal control (group A). A concentration-dependent increase in the catalase activity in the gills of the extracts treated groups was observed. The negative control had elevated levels of the catalase activity in the gills when compared with the standard drug (group C), extracts treated groups (D - G) and the normal control. The increase is not statistically significant ($p >0.05$). Comparing groups with their baselines (before treatment), there was a reduced catalase activity at the end of the 96h in the negative control, and the treated groups. The decrease was time dependent and dose independent. Comparing the groups along the column, no significant differences ($p > 0.05$) were observed in the catalase activity of the gills before treatment and throughout the short-term treatment (24-96h). (Table 55).

The catalase activity in the muscle of the ich infested *C. gariepinus* among the extracts treated groups (D - G) at the end of the short term treatments (96h) was highest in group D and F when compared to the lower activity recorded in group E and G which maintained similar catalase activity levels in the muscle. There was no significant differences ($p > 0.05$) in the increased and decreased catalase activities recorded among groups D and F and between E and G. Among these, the catalase activities did not increase or decrease in a dose-dependent manner.

Meanwhile, the negative control had elevated levels of the catalase activity in the muscle when compared with the standard drug and extracts treated groups. The increase in the catalase activity in the negative control was significant ($p < 0.05$) compared to the normal control and the treated groups. Comparing each group with their baselines (before treatment), there were reduced catalase activities at the end of the 96h in the negative control and the treated groups. The decrease was time dependent and dose independent. There were no significant differences ($p > 0.05$) in the catalase activities in the muscles among the groups along the column before treatment and 24 - 72h. Moreover, a significant differences ($p < 0.05$) were observed in the catalase activities in the muscle among the groups along the column at the end of 96h (Table 55).

Table 55: Changes in the catalase activity (µmol of H_2O decomposed/min/mg protein) in the gills and muscles of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Tissue	Before treatment	24h	48h	72h	96h
A	Gill	1.05 ± 0.17^{a1}	1.01 ± 0.18^{a1}	0.69 ± 0.07^{a1}	2.36 ± 1.42^{b2}	1.25 ± 1.01^{a1}
	Muscle	1.08 ± 0.06^{a1}	0.89 ± 0.04^{ab1}	$0.38 \pm 0.45^{a2,1}$	1.05 ± 0.97^{a1}	0.07 ± 0.06^{a2}
B	Gill	1.60 ± 0.96^{a1}	1.09 ± 0.12^{a1}	0.819 ± 0.11^{a1}	1.37 ± 0.76^{a1}	0.8 ± 0.05^{a1}
	Muscle	1.61 ± 0.29^{a1}	0.57 ± 0.31^{a1}	0.77 ± 0.11^{b1}	0.74 ± 0.04^{a1}	1.29 ± 0.53^{b1}
C	Gill	2.45 ± 1.62^{a1}	1.01 ± 0.13^{a1}	0.56 ± 0.45^{a2}	0.36 ± 0.40^{a2}	0.71 ± 0.05^{a2}
	Muscle	1.58 ± 0.39^{a1}	0.68 ± 0.19^{ab1}	0.77 ± 0.06^{b1}	0.82 ± 0.04^{a1}	0.72 ± 0.05^{a1}
D	Gill	1.72 ± 1.98^{a1}	1.36 ± 0.92^{a1}	0.77 ± 0.05^{a1}	0.89 ± 0.13^{a1}	0.75 ± 0.04^{a1}
	Muscle	2.80 ± 1.18^{b1}	0.92 ± 0.22^{ab2}	0.84 ± 0.06^{b2}	0.73 ± 0.03^{a2}	0.75 ± 0.04^{a2}
E	Gill	1.79 ± 1.88^{a1}	1.02 ± 0.25^{a1}	0.74 ± 0.2^{a1}	0.58 ± 0.04^{a1}	0.76 ± 0.49^{a1}
	Muscle	1.53 ± 0.45^{a1}	0.89 ± 0.26^{ab1}	0.81 ± 0.06^{b1}	0.81 ± 0.12^{a1}	0.74 ± 0.03^{a1}
F	Gill	2.51 ± 0.98^{a1}	0.88 ± 0.17^{a1}	0.79 ± 0.03^{a1}	0.92 ± 0.25^{a1}	0.79 ± 0.08^{a1}
	Muscle	1.64 ± 0.27^{a1}	1.00 ± 0.12^{b1}	0.81 ± 0.15^{b1}	0.75 ± 0.03^{a1}	0.75 ± 0.02^{a1}
G	Gill	2.61 ± 1.59^{a1}	1.55 ± 0.75^{a1}	0.78 ± 0.06^{a1}	1.02 ± 0.43^{a1}	0.84 ± 0.08^{a1}
	Muscle	1.66 ± 0.36^{a1}	0.84 ± 0.19^{ab1}	0.78 ± 0.2^{b1}	0.81 ± 0.03^{a1}	0.74 ± 0.03^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

3.17.3. Changes in the catalase activity in the gills and muscles of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 56 showed that the catalase activity in the ich infested *C. gariepinus* among the extracts treated groups increased in day 15 in group F treated with 3,500mg/L when compared with its baseline (before treatment). At the same time, group F that had the highest level of catalase activity in the gills equally had an elevated level of the catalase in day 7 which further reduced thereafter at the end of the prolonged bath treatment. However, the increased catalase activity in the group F is significantly different ($p < 0.05$) when compared with the control. Furthermore, similar mixed trends in the activities of the catalase in the gills were equally observed among other groups. Though, when their mean values were compared with baselines, increased significant ($p < 0.05$) catalase activities were established. It is worthy to note that the catalase activity in the gills of the group B had a marked increase throughout the duration of the prolonged bath treatments. At the same time, the normal control maintained a significantly ($p < 0.05$) reduced catalase activity in the gills. The catalase activity in the gills among the groups in the column did not differ significantly ($p > 0.05$) before treatments, but differed significantly ($p < 0.05$) in day 7 and day 15 during the prolonged bath treatment. The standard drug had the lowest catalase activity level when compared to the extractss groups. The changes in the catalase activity of the muscle differed significantly ($p < 0.05$) among the groups in the column, before treatment and day 7 but did not differ significantly at $p >0.05$ in day 15. Mixed trends in the catalase activities level were observed among the groups whereas only groups B and D maintained a steady increase in the catalase activity in the muscle with group B having the highest elevation. Catalase activity in each group had an elevated level at the end of the

prolonged bath treatments when compared with there baselines that had reduced catalase activities in the muscle of the ich infested C. *gariepinus* (Table 56).

Table 56: Changes in the catalase activity (µmol of H_2O decomposed/min/mg protein) in the gills and muscles of *Clarias gariepinus* after prolonged bath treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Tissue	Before Treatment	Day 7	Day 15
A	Gill	1.25 ± 0.12^{a1}	2.90 ± 0.64^{a1}	2.45 ± 0.59^{a1}
	Muscle	1.76 ± 0.89^{ab1}	1.02 ± 0.20^{a1}	10.21 ± 5.74^{b2}
B	Gill	0.67 ± 0.43^{a1}	3.19 ± 1.98^{a2}	7.76 ± 3.55^{bc3}
	Muscle	1.56 ± 0.21^{ab1}	6.67 ± 6.39^{ab2}	10.94 ± 7.28^{b3}
C	Gill	1.23 ± 1.68^{a1}	2.76 ± 1.75^{a2}	5.69 ± 2.17^{ab3}
	Muscle	2.91 ± 1.33^{b1}	7.24 ± 1.05^{ab2}	5.98 ± 2.41^{a1}
D	Gill	1.39 ± 0.66^{a1}	10.28 ± 1.09^{ab2}	9.05 ± 1.94^{bc2}
	Muscle	1.76 ± 0.44^{ab1}	6.64 ± 2.95^{ab2}	7.1 ± 5.00^{a2}
E	Gill	0.58 ± 0.74^{a1}	17.66 ± 3.54^{bc2}	7.41 ± 0.97^{bc2}
	Muscle	1.37 ± 0.87^{a1}	10.26 ± 1.09^{b2}	11.18 ± 4.63^{c2}
F	Gill	1.25 ± 0.45^{a1}	15.94 ± 6.69^{b2}	10.96 ± 2.01^{c2}
	Muscle	1.08 ± 0.27^{a1}	12.17 ± 2.73^{b2}	11.33 ± 1.11^{a3}
G	Gill	1.29 ± 0.77^{a1}	24.02 ± 7.74^{c2}	9.92 ± 2.10^{c3}
	Muscle	1.03 ± 0.84^{a1}	20.13 ± 8.16^{c2}	10.44 ± 1.80

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

3.17.4 Changes in the superoxide dismutase (SOD) in the gills and muscles tissues of *Clarias gariepinus* during dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Mean values of SOD in the gills of Ich infested groups were comparable to those in the control group after 1h treatments. There were no significant differences ($p > 0.05$) among the Ich infested groups (B - G) when compared to the normal control group. At the same time among the extracts treated groups, the mean values of SOD were higher in the group G when compared to other groups (D - F) and the positive control. Comparing the mean values among the groups along the column, there were no significant differences ($p > 0.05$) in SOD levels in the gills of the ich infested fish among the groups before treatments and end of the dip treatments (Table 57)

The SOD activity in the muscle of the ich infested *C. gariepinus* differed significantly ($p < .0.5$) among the ich infested groups and the normal control before treatment and was not statistically significant ($p > 0.05$) at the end of the dip treatment (Table 57).

Table 57: Changes in the superoxide dismutase (SOD) ((µmol of H_2O decomposed/min/mg protein) in the gill and muscle tissues of *C. gariepinus* during dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Exp. Group	Tissue	Before treatment	After treatment
A	Gill	82.68 ± 16.33^{a}	64.01 ± 7.24^{a}
	Muscle	56.69 ± 7.78^{a}	73.06 ± 7.47^{a}
B	Gill	89.78 ± 10.41^{a}	80.0 ± 8.65^{b}
	Muscle	84.26 ± 4.57^{b}	81.96 ± 2.00^{a}
C	Gill	80.38 ± 1.99^{a}	77.04 ± 41.18^{b}
	Muscle	81.08 ± 1.85^{b}	80.56 ± 2.42^{a}
D	Gill	83.91 ± 3.62^{a}	78.36 ± 3.49^{b}
	Muscle	95.84 ± 15.33^{b}	81.02 ± 7.02^{a}
E	Gill	84.78 ± 5.13^{a}	80.36 ± 5.53^{b}
	Muscle	85.54 ± 11.79^{b}	77.08 ± 54.49^{a}
F	Gill	79.51 ± 4.13^{a}	74.48 ± 9.46^{b}
	Muscle	90.63 ± 10.04^{b}	81.44 ± 4.28^{a}
G	Gill	82.74 ± 5.69^{a}	85.71 ± 7.89^{b}
	Muscle	96.23 ± 13.21^{b}	81.98 ± 8.56^{a}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different alphabets as superscript in a column are significantly different ($P<0.05$). A (control), B (infected not treated), C (infected treated with standard drug), D (1500mg/L), E (2500mg/L), F(3500mg/L) and G (4500mg/L).

3.17.5. Changes in the superoxide dismutase (SOD) in the gills and muscles tissues of *Clarias gariepinus* during short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Mean values of SOD in the infested group were not comparable to those in the control group (normal control) before the treatment commenced and SOD activities in the gills were not statistically significant ($p > 0.05$) when compared to the control. During the treatment periods, in 24h the SOD levels were higher in the group A (Normal control) compared to the treated groups and negative control. However, no significant changes ($p > 0.05$) were observed among the groups throughout the treatment periods. At the same time, considering the extracts treated groups and the control (Negative and Positive) at the end of short term treatments, the SOD levels in the gills were reduced but the reduction did not differ significantly ($p > 0.05$) when compared to their baselines (before treatment) (Table 58).

The SOD activity in the muscle was not comparable to those in the control group (Negative control) before the treatment commenced. During the treatment periods, in 24h, the SOD levels were higher in the group A compared to the treated groups and negative control. No significant changes ($p > 0.05$) were observed among the groups throughout the short term treatments except in 72h period of treatment that differed significantly ($p < 0.05$) with the negative control eliciting higher SOD levels in comparison with the treated groups and normal control. (Table 58).

Tables 58: Changes in the superoxide dismutase (SOD) (µmol of H_2O decomposed/min/mg protein) in the gills and muscles of *C. gariepinus* during short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Exp group	Tissue	Before	24h	48h	72h	96h
A	Gill	71.05 ±8.51^{a1}	86.57 ± 9.25^{b1}	70.81 ± 5.30^{a1}	66.9 ± 7.74^{a1}	69.22 ± 4.33^{a1}
	Muscle	72.34 ± 8.01^{a1}	89.00 ± 10.51^{b1}	71.35 ± 5.59^{a1}	72.41 ± 1.74^{ab1}	71.31 ± 1.71^{a1}
B	Gill	80.98 ± 7.15^{a1}	66.05 ± 11.25^{b1}	72.73 ± 5.07^{a1}	86.69 ± 12.06^{b1}	76.02 ± 1.92^{ab1}
	Muscle	87.29 ± 6.22^{ab1}	74.89 ± 8.56^{ab}	75.80 ± 3.12^{a1}	35.08 ± 7.06^{b1}	76.92 ± 3.27^{b1}
C	Gill	82.38 ± 6.89^{a1}	76.58 ± 5.63^{ab1}	85.04 ± 5.71^{b1}	69.82 4.87^{ab1}	71.88 ± 1.82^{ab1}
	Muscle	80.97 ± 4.01^{ab1}	70.53 ± 5.96^{a1}	76.35 ± 4.59^{a1}	68.56 ± 4.94^{a1}	72.53 ± 1.19^{a1}
D	Gill	82.76 ± 14.51^{a1}	76.18 ± 4.21^{ab1}	73.77 ± 4.56^{a1}	74.45 ± 1.77^{ab1}	74.88 ± 5.18^{ab1}
	Muscle	82.59 ± 5.75^{a1}	63.27 ± 13.32^{a1}	73.06 ± 4.88^{a1}	7.05 ± 1.78^{b1}	71.29 ± 0.88^{a1}
E	Gill	80.14 ± 7.98^{a1}	75.86 ± 2.58^{ab1}	67.58 ± 4.41a1	82.25 ± 5.60^{b1}	78.86 ± 5.51^{b1}
	Muscle	91.17 ± 16.62^{ab1}	69.97 ± 7.63^{a1}	76.65 ± 2.40^{a1}	77.92 ± 3.11^{ab1}	72.13 ± 2.73^{a1}
F	Gill	90.61 ± 17.07^{a1}	77.15 ± 2.53^{ab1}	73.48 ± 3.84^{a1}	72.92 ± 1.41^{ab1}	75.17 ± 2.93^{ab1}
	Muscle	92.85 ± 14.76^{b1}	78.94 ± 2.52^{ab1}	75.98 ± 5.42^{a1}	77.17 ± 3.92^{b1}	71.55 ± 1.49^{a1}
G	Gill	91.78 ± 12.12^{a1}	61.65 ± 26.56^{a1}	77.44 ± 6.62^{ab1}	80.91 ± 4.98^{b1}	75.17 ± 5.58^{ab1}
	Muscle	82.89 ± 5.60^{ab1}	71.47 ± 5.88^{a1}	74.76 ± 4.58^{a}	77.24 ± 3.56^{b}	73.89 ± 3.06^{ab1}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different numbers as superscript in a rowcolumn are significantly different (P<0.05). A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L)

3.17.6. Changes in the SOD in the gills and muscle of *C. gariepinus* during prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Mean values of SOD in the Ich infested group were not comparable to those in the control group (Normal control) before the treatment commenced and the SOD activities in the gills were not statistically significant ($p > 0.05$) when compared to the control. However, during the treatment periods (day 7 and day15) the SOD levels in the gills differed significantly ($p < 0.05$) when compared to the control. Furthermore, the mean values of SOD activities in the gills among the extracts treated groups reduced over time throughout the treatment period when compared to the baseline of each group. The decrease was time-dependent and concentration independent. The decrease in SOD level recorded in the positive control was comparable to the SOD levels in the normal control whereas the SOD level in the negative control (group B) increased over time compared to the treated groups that had reduced SOD levels. (Table 59)

The SOD activities in the muscle were fairly comparable to those in the control group (normal control) before commencement of the treatment. However, the SOD activities in the muscle of the ich infested fish among the groups was not statistically significant ($p > 0.05$) when compared to the control. During the treatment periods in day 7 and day 15, the SOD levels in the negative control were higher compared to the extracts and standard drug treated groups and the increase was statistically significant ($p < 0.05$). The mean values of SOD activities in the muscle among the extracts treated groups and the standard drug group reduced over time throughout the treatment period when compared to the baseline of each group. The decrease was time-dependent and concentration independent. The SOD levels in the negative control were higher throughout the treatment period when compared to the treated groups whereas at the end of the prolonged

bath treatment, significant SOD level was seen in the normal control when compared to all the groups (B-G) (Table 59).

Table 59: Changes in the superoxide dismutase (µmol of H_2O decomposed/min/mg protein) in the gill and muscle tissues of *Clarias gariepinus* during prolonged bath treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Expermental group	Tissue	Before treatment	Day 7	Day 15
A	Gill	75.64 ± 13.61^{a1}	20.50 ± 9.30^{a2}	28.20 ± 8.17^{a2}
	Muscle	71.53 ± 2.84^{a1}	74.54 ± 23.19^{bc1}	93.89 ± 24.39^{c1}
B	Gill	83.00 ± 15.57^{a1}	73.46 ± 15.21^{d1}	71.95 ± 11.09^{c1}
	Muscle	86.65 ± 11.93^{a1}	82.63 ± 15.81^{c1}	88.20 ± 14.48^{ab1}
C	Gill	79.55 ± 10.69^{a1}	49.66 ± 12.44^{bc2}	21.41 ± 13.34^{a3}
	Muscle	91.65 ± 12.36^{a1}	52.99 ± 18.67^{a2}	47.07 ± 14.20^{ab3}
D	Gil	77.98 ± 11.40^{a1}	33.99 ± 14.00^{b2}	$44.49.7.28^{b2}$
	Muscle	88.34 ± 8.47^{a1}	63.59 ± 27.33^{ab1}	54.70 ± 11.87^{ab2}
E	Gill	78.18 ± 32.69^{a1}	53.81 ± 19.51^{bc2}	36.32 ± 6.92^{ab3}
	Muscle	78.74 ± 13.43^{a1}	66.60 ± 20.39^{abc1}	56.65 ± 11.78^{a1}
F	Gill	31.16 ± 27.17^{a1}	64.99 ± 15.99^{cb2}	38.32 ± 13.56^{ab1}
	Muscle	74.73 ± 16.69^{a1}	67.61 ± 22.62^{abc1}	45.41 ± 18.89^{ab2}
G	Gill	81.33 ± 12.3^{a1}	53.31 ± 13.95^{bc2}	36.88 ± 5.99^{ab3}
	Muscle	84.16 ± 13.7^{5a1}	54.12 ± 18.24^{a2}	51.24 ± 9.50^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$).). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

3.17.7. Changes in the gluthathione peroxidase (U/mg protein) in the gills and muscles of *Clarias gariepinus* during dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Mean values of GPx in the Ich infested group were not comparable to those in the control group (normal control) before the treatment commenced and the GPx activities in the gills were not statistically significant ($p > 0.05$) when compared to the control. The GPx activities in the gills of the infested fish were higher in the negative control at the end of the dip treatments when compared to the treated groups and the normal control. The increase in GPx activity recorded in the negative control did not differ significantly ($p > 0.05$) with other groups. The GPx activity in the groups along the column did not differ significantly ($p > 0.05$) before and after treatments. Furthermore, there is a fairly reduced GPx activity in the gills of each group when compared to their corresponding baselines (before treatment) (Table 60).

The GPx activity in the muscle of the ich infested *C. gariepinus* did not differ significantly ($p > 0.05$) among the groups before and after treatments. Moreover, the normal control maintained a fairly reduced GPx activity when compared to the elevated levels of the GPx activity in the muscle of the treated groups and the negative control while comparing means along the column before and after treatment. Therefore, comparing the GPx activity in the muscles of each group to their baselines (before treatment), showed a fairly increased GPx activities in the muscle of some treated groups C D F and G) whereas some had a fairly reduced GPx activity in the gill and was not significantly different ($p > 0.05$) (Table 60).

Table 60: Changes in gluthathione peroxidase (U/mg protein) of gill and muscle of *Clarias gariepinus* during dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental Group	Tissues	Before Treatment	After Treatment
A	Gill	27.67 ± 55.36^{a1}	24.71 ± 4.76^{a1}
	Muscle	24.89 ± 1.36^{a1}	37.78 ± 4.09^{a1}
B	Gill	35.34 ± 6.07^{a1}	37.24 ± 6.28^{b1}
	Muscle	39.70 ± 6.79^{b1}	46.15 ± 12.15^{a1}
C	Gill	34.06 ± 2.76^{a1}	29.91 ± 4.54^{ab1}
	Muscle	37.23 ± 5.09^{ab1}	44.36 ± 16.99^{a1}
D	Gill	35.31 ± 2.10^{ab1}	29.06 ± 2.11^{ab1}
	Muscle	41.62 ± 9.51^{b1}	41.95 ± 9.96^{a1}
E	Gill	34.17 ± 2.97^{ab1}	32.70 ± 6.57^{ab1}
	Muscle	40.46 ± 10.17^{b1}	39.27 ± 1077^{ab1}
F	Gill	39.86 ± 8.69^{b1}	32.58 ± 5.03^{ab1}
	Muscle	41.31 ± 8.10^{b1}	46.15 ± 15.33^{a1}
G	Gill	34.32 ± 9.81^{ab1}	32.77 ± 1.94^{ab1}
	Muscle	34.74 ± 4.13^{ab1}	41.71 ± 10.48^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$).). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

3.17.8 Changes in the gluthathione peroxidase (U/mg protein) of gill and muscle of *Clarias gariepinus* during short term treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 61 shows that mean values of GPx in the gills of ich infested group were not comparable to those in the control group (Normal control) before the treatment. The GPx activity in the gills were not statistically significant ($p > 0.05$). During the treatment period in 24h and 48h while comparing means along the column, there were significant differences at $p > 0.05$ among the GPx activities in the gills among the groups, whereas, at 72h and 96h, there were significant differencse ($p<0.05$) in the GPx activities in the gills of Ich infested *C. gariepinus* among the groups. Considering the extracts treated groups and the control (negative and positive) at the end of the short term treatments, the GPx levels in the gills reduced but the reduction differed significantly ($p > 0.05$) when compared to their baselines (before treatment)

The GPx activity in the muscles was comparable to those in the control group (Normal control) before treatment commenced. The GPx activities in the muscle of the Ich infested fish among the groups were not statistically significant ($p > 0.05$) when compared to group A. During the treatment period in 24h and 48h while comparing means along the column there were no significant differences at ($p > 0.05$) among the GPx activities in the muscles of the infested fish among the groups. Moreso, at 72h and 96h, there were significant differences ($p < 0.05$) in the GPx activities in the muscles of ich infested *C. gariepinus* among the groups. While considering the extracts treated groups and the control (negative and positive) at the end of the short term treatments, the GPx levels in the muscle were reduced and the decrease were not statistically significant ($p > 0.05$) when compared to their baseline (before treatment), whereas, a different

trend was observed in the group A (normal control) which had fairly elevated level of GPx at the end of the short term treatment when compared to its baseline (before treatment) (Table 61).

Table 61: Changes in GPx (U/mg protein) activity in the gills and muscles of *Clarias gariepinus* during short term treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Tissue	Before treatment	24h	48h	72h	96h
A	Gill	24.48 ± 5.96^{a1}	30.56 ± 2.28^{a1}	27.12 ± 4.4^{a1}	23.30 ± 44.47^{a1}	28.40 ± 2.41^{a1}
	Muscle	21.88 ± 2.55^{a1}	39.84 ± 2.05^{b1}	26.98 ± 6.63^{a1}	29.83 ± 1.61^{a1}	35.37 ± 2.49^{d1}
B	Gill	37.18 ± 36.30^{a1}	29.94 ± 1.59^{a1}	30.95 ± 0.71^{a1}	35.86 ± 4.18^{c1}	30.98 ± 2.93^{ab1}
	Muscle	36.96 ± 13.76^{b1}	30.00 ± 2.64^{ab1}	33.88 ± 1.05^{a1}	29.67 ± 1.77^{a1}	26.1 ± 3.01^{a1}
C	Gill	43.15 ± 12.73^{a1}	31.49 ± 11.46^{a1}	32.10 ± 4.31^{b1}	27.09 ± 5.16^{ab1}	30.07 ± 0.61^{a1}
	Muscle	30.98 ± 3.79^{b1}	30.77 ± 2.07^{a1}	29.79 ± 1.07^{a1}	23.83 ± 5.09^{a1}	29.67 ± 2.28^{abc1}
D	Gil	42.49 ± 7.21^{a1}	31.59 ± 12.26^{a1}	32.44 ± 7.47^{a1}	31.06 ± 1.58^{bc1}	31.31 ± 1.58^{ab1}
	Muscle	38.79 ± 4.71^{b1}	24.00 ± 6.10^{a1}	35.57 ± 3.36^{a1}	31.47 ± 1.51^{a1}	30.81 ± 0.55^{bc1}
E	Gill	42.18 ± 6.87^{a1}	29.28 ± 2.83^{a1}	37.48 ± 2.42^{ab1}	30.59 ± 1.77^{bc1}	31.54 ± 1.72^{ab1}
	Muscle	34.98 ± 6.03^{b1}	32.32 ± 2.77^{b1}	35.91 ± 5.46^{a1}	30.83 ± 1.22^{a1}	31.55 ± 1.21^{c1}
F	Gill	48.29 ± 20.49^{a1}	30.26 ± 1.09^{a1}	37.87 ± 2.42^{a1}	30.79 ± 2.05^{bc1}	33.57 ± 1.58^{bc1}
	Muscle	34.62 ± 39.95^{b1}	30.92 ± 1.94^{ab1}	28.84 ± 2.49^{a1}	30.41 ± 2.24^{a1}	31.08 ± 0.57^{b1}
G	Gill	45.89 ± 16.96^{a1}	31.29 ± 1.04^{a1}	32.47 ± 2.51^{ab1}	31.61 ± 1.67^{bc1}	36.24 ± 1.28^{c1}
	Muscle	36.78 ± 3.89^{b1}	30.58 ± 1.66^{ab1}	26.87 ± 5.26^{a1}	31.82 ± 1.75^{a1}	26.97 ± 3.08^{ab1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$).). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

3.17.9. Changes in the gluthathione peroxidase (U/mg protein) in the gills and muscles of *Clarias gariepinus* during prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 62 Shows that the mean values of GPx in the gills of ich infested fish were comparable to those in the control group (Normal control) before the treatment commenced. Moreso, the GPx activity in the gills were statistically significant ($p < 0.05$) when compared to the control along the column. In Day 7, the GPx activity in the gills of the ich infested *C. gariepinus* among the groups did not differ significantly ($p > 0.05$) although the activity in group A decreased when compared to other groups. No significant differences ($p > 0.05$) were observed in the GPx activity in the gill in day 7 but they differed significantly ($p < 0.05$) at the end of the prolonged bath treatments among the groups when compared to the control (group A.)

The GPx activity in the muscle of the Ich infested *C. garipinus* was comparable to the normal control before the commencement of the treatment. At the same time, there were no significant differences ($p > 0.05$) among the groups when compared to the control. At the same time, there was significant difference ($p < 0.05$) in day 7 among the groups along the column which did not differ significantly at the end of the prolonged bath treatments ($p > 0.05$) (Table 62).

Table 62: Changes in the GPx (U/mg protein) activity in the gills and muscles of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Tissue	Before treatment	Day 7	Day 15
A	Gill	28.64 ±1.44^{a1}	18.25 ±6.60^{a2}	17.44 ±4.02^{a2}
	Muscle	26.00 ± 4.07^{a1}	29.25 ± 4.52^{ab1}	49.69 ± 9.07^{a2}
B	Gill	36.20 ±4.57^{bc1}	37.58 ±12.51^{a1}	34.35 ±4.59^{bc1}
	Muscle	36.68 ±3.16^{b1}	31.47 ±2.22^{abc1}	34.92 ±11.15^{a1}
C	Gill	35.72 ±5.24^{abc1}	37.58 ±12.51^{a1}	28.73 ±9.32^{ab1}
	Muscle	36.99 ±5.06^{b1}	21.28 ±2.72^{a1}	23.59 ±2.17^{b1}
D	Gill	33.01 ±2.02^{ab1}	32.26 ±7.78^{a1}	40.67 ±10.65^{bc1}
	Muscle	40.53 ±1.47^{b1}	38.03 ±3.42^{bc1}	34.67 ±6.56^{a1}
E	Gill	41.42 ±2.05^{c1}	38.03 ±3.42^{bc1}	42.69 ±6.03^{c1}
	Muscle	38.20 ±3.129^{b1}	43.77 ±14.67^{c1}	36.69 ±4.91^{a1}
F	Gill	39.64 ±12.58^{b1}	26.57 ±10.99^{a1}	39.23 ±1.05^{bc1}
	Muscle	38.08 ±4.40^{bc1}	32.19 ± 11.57^{a1}	40.08 ±9.99^{a1}
G	Gill	31.44 ±2.12^{ab1}	37.50 ±8.07^{bc1}	37.86 ±4.07^{bc1}
	Muscle	31.44 ±2.12^{ab1}	37.50 ±8.07^{bc1}	38.92 ±9.44^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05).). Mean values with different numbers as superscript in a row are significantly different (P<0.05). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L).

3.18. Changes in the malondialdehyde (MDA) activities in the gill and muscle tissues of *Clarias gariepinus* during dip, short term and prolonged bath treatments with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

3.18.1 Changes in the MDA activities in the gill and muscle tissues of *Clarias gariepinus* during dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 63 shows that MDA levels in the extracts treated groups were higher in the group E and lower in group G whereas the increase and decrease observed were not significantly different ($p > 0.05$) when compared to the normal control. At the same time, there were variations in the MDA levels among the groups especially when comparing the post treatment with the pre-treatment during the dip treatments. The MDA levels before treatment when compared with the groups along the column differed significantly ($p < 0.05$) but were not significantly different ($p > 0.05$) at the end of the dip treatments. However, the MDA levels in the normal control at the end of the dip treatments were lower when compared to other treated groups and the negative controls.

The MDA levels in the muscles when compared among the groups along the column did not vary significantly ($p > 0.05$) after 1h treatments. Meanwhile before treatment, the MDA levels were lower in the group A compared to other Ich infested groups and the decrease differed significantly ($p < 0.05$). (Table 63).

Table 63: Changes in the malondialdehyde (MDA) (nmol of MDA formed/mg protein) activities in the gill and muscle tissues of *Clarias gariepinus* during dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Tissue	Before treatment	After treatment (1h)
A	Gill	14.97 ± 1.52^{b1}	6.61 ± 0.48^{a2}
	Muscle	2.87 ± 0.59^{a1}	7.35 ± 2.08^{a1}
B	Gill	7.49 ± 1.69^{a1}	9.16 ± 1.93^{a1}
	Muscle	9.50 ± 3.54^{b1}	10.35 ± 2.69^{a1}
C	Gill	8.20 ± 2.83^{a1}	7.44 ± 0.83^{a1}
	Muscle	10.21 ± 2.65^{b1}	7.07 ± 2.00^{a1}
D	Gill	8.83 ± 2.96^{a1}	9.44 ± 0.88^{a1}
	Muscle	11.12 ± 4.27^{b1}	9.51 ± 4.57^{a1}
E	Gill	9.35 ± 3.69^{a1}	11.37 ± 5.93^{a1}
	Muscle	8.89 ± 3.30^{b1}	12.89 ± 6.18^{a1}
F	Gill	7.39 ± 1.72^{a1}	8.88 ± 1.71^{a1}
	Muscle	9.62 ± 3.05^{a1}	11.99 ± 4.84^{a1}
G	Gill	8.74 ± 2.77^{a1}	8.39 ± 1.06^{a1}
	Muscle	9.01 ± 2.61^{b1}	10.76 ± 4.18^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$).). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L).

3.18.2 Changes in the malondialdehyde (MDA) activities in the gill and muscle tissues of *Clarias gariepinus* during short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 64 shows the mean variations in the MDA activities in the gills. When the groups were compared along the column, the MDA activity in the gills did not differ significantly ($p > 0.05$) before treatments in 24 h, 72 h and 96 h. But there was significant difference ($p < 0.05$) in the MDA activity in the gills in 48h among the groups. The MDA activity in the gills of the infested fish in each groups had a mixed trend throughout the short term treatments. Though the changes in their MDA activities were not time-dependent.

Furthermore, the MDA activity in the muscles, when compared with the groups along the column was not significantly different ($p > 0.05$) before treatments in 24 h, 48 h and 96 h treatments but it differed significantly in 72h treatment ($p < 0.05$). When the standard drug and extracts groups were compared, the MDA level in the group C (standard drug) was lower than the extracts treated groups but did not differ significantly ($p > 0.05$) (Table 64).

Table 64: Changes in the malondialdehyde (MDA) (nmol of MDA formed/mg protein) in the gill and muscle tissues of *Clarias gariepinus* during short term treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Tissue	Before treatment	24h	48h	72h	96h
A	Gill	4.82 ± 1.46^{a1}	7.84 ± 1.23^{a1}	6.25 ± 0.86^{a1}	6.67 ± 1.61^{a1}	7.19 ± 0.83^{a1}
	Muscle	5.78 ± 2.31^{a1}	5.96 ± 0.49^{a1}	7.03 ± 0.60^{a1}	7.53 ± 0.06^{abc1}	7.08 ± 0.66^{a1}
B	Gill	9.76 ± 3.13^{ab1}	7.92 ± 0.61^{a1}	7.03 ± 0.34^{ab1}	9.15 ± 2.08^{a1}	7.99 ± 0.34^{ab1}
	Muscle	10.33 ± 3.62^{ab1}	8.47 ± 0.89^{b1}	8.83 ± 0.95^{a1}	7.92 ± 0.57^{bc1}	8.37 ± 0.73^{a1}
C	Gill	8.08 ± 1.46^{ab1}	7.54 ± 0.26^{a1}	7.57 ± 0.07^{b1}	9.27 ± 1.94^{a1}	7.58 ± 0.43^{ab1}
	Muscle	8.69 ± 2.79^{ab1}	8.06 ± 0.84^{b1}	8.68 ± 1.32^{a1}	7.47 ± 0.16^{abc1}	7.40 ± 0.89^{a1}
D	Gill	8.80 ± 2.13^{ab1}	9.53 ± 2.47^{a1}	7.53 ± 0.19^{b1}	8.64 ± 1.39^{a1}	9.29 ± 1.50^{a1}
	Muscle	9.96 ± 2.35^{ab1}	7.59 ± 0.28^{ab1}	7.98 ± 1.59^{a1}	7.89 ± 0.30^{bc1}	9.02 ± 1.47^{a1}
E	Gill	9.11 ± 2.13^{ab1}	10.59 ± 3.15^{a1}	7.59 ± 0.64^{b1}	10.12 ± 2.24^{a1}	8.75 ± 1.29^{ab1}
	Muscle	12.15 ± 4.19^{b1}	8.81 ± 2.01^{b1}	7.90 ± 0.27^{a1}	8.34 ± 0.69^{c1}	8.56 ± 1.51^{a1}
F	Gill	10.26 ± 3.00^{ab1}	11.46 ± 3.77^{a1}	7.64 ± 0.46^{b1}	7.08 ± 6.39^{a1}	8.07 ± 0.79^{ab1}
	Muscle	10.58 ± 2.92^{ab1}	7.68 ± 0.89^{b1}	8.65 ± 0.85^{a1}	7.21 ± 0.35^{ab1}	8.39 ± 0.94^{a1}
G	Gill	12.07 ± 4.03^{b1}	8.34 ± 1.05^{a1}	7.54 ± 0.56^{b1}	10.20 ± 3.70^{a1}	8.17 ± 0.92^{ab1}
	Muscle	9.07 ± 1.99^{ab1}	8.26 ± 0.26^{b1}	8.14 ± 0.56^{a1}	6.86 ± 0.49^{a1}	8.40 ± 0.82^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F(350mg/L) and G (450mg/L).

3.18.3 Changes in the malondialdehyde (MDA) (nmol of MDA formed/mg protein) in the gill and muscle tissues of *C. gariepinus* during prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 65 Shows that the MDA levels in the gills of the groups at the end of the prolonged bath treatments were lowest in the group B (negative control) when the means among the treated groups were compared. The group C had the lowest MDA level at the end of the day 7 when compared to the extractss groups that had highest MDA levels in group D although the increase among the extracts groups were dose independent. At the same time the group G had a steady increase in their MDA levels with peak level in day 15.In like manner, group D had similar trend when compared to other treated groups that had mixed trends in their MDA levels. The MDA activity in the gills among the groups did not differ significantly ($p > 0.05$) throughout the prolonged bath treatments and pre-treatments.

The MDA activity in the muscle of the Ich infested *C. gariepinus* differed significantly ($P > 0.05$) when the groups along the column were compared before treatments and throughout the prolonged bath treatments (Table 65). Meanwhile before treatments, the MDA levels in the group A were lower when compared to other ich infested *C. gariepinus* groups. The decrease differed significantly ($p < 0.05$) when compared to other Ich infested groups.

Table 65: Changes in the Malondialdehyde (MDA) (nmol of MDA formed/mg protein) in the gill and muscle tissues of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Tissue	Before	Day 7	Day 15
A	Gill	9.00 ± 2.48^{a1}	12.69 ± 2.09^{a1}	19.82 ± 7.79^{abc2}
	Muscle	4.13 ± 1.41^{a1}	7.57 ± 0.89^{a1}	9.62 ± 1.37^{a1}
B	Gill	7.76 ± 2.07^{a1}	12.93 ± 5.68^{a1}	8.05 ± 0.53^{a1}
	Muscle	7.43 ± 0.08^{b1}	8.11 ± 0.98^{a1}	8.23 ± 0.57^{a1}
C	Gill	8.10 ± 1.00^{a1}	24.71 ± 6.77^{a2}	13.08 ± 5.51^{ab1}
	Muscle	8.76 ± 2.07^{a1}	18.34 ± 3.60^{ab1}	15.69 ± 6.37^{b1}
D	Gill	7.15 ± 0.23^{a1}	18.97 ± 6.68^{a2}	29.06 ± 7.55^{c2}
	Muscle	7.32 ± 1.03^{b1}	17.16 ± 7.45^{ab2}	18.98 ± 1.54^{b2}
E	Gill	7.13 ± 1.03^{a1}	18.96 ± 10.54^{a2}	19.11 ± 11.94^{ab2}
	Muscle	10.97 ± 1.55^{c1}	20.13 ± 1033^{bc2}	19.15 ± 1.70^{b2}
F	Gill	8.71 ± 1.46^{a1}	20.02 ± 2.09^{a2}	19.14 ± 2.29^{abc2}
	Muscle	8.70 ± 63^{ab1}	20.29 ± 3.2^{bc2}	14.12 ± 4.29^{b1}
G	Gill	7.86 ± 0.85^{a1}	17.94 ± 7.10^{a2}	25.62 ± 9.96^{bc2}
	Muscle	8.68 ± 2.15^{ab1}	30.26 ± 9.12^{c2}	26.99 ± 6.82^{c2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F(35mg/L) and G (45mg/L).

3.19. Changes in the Myeloperoxidase (1 µmol H_2O_2/min) Activity in the Gills and Muscles of *Clarias gariepinus* after Dip, Short Term and Prolonged bath Treatments with Aqueous Extracts of *Moringa oleifera* Leaves and Standard Drug (fish cure).

3.19.1. Changes in the myeloperoxidaxe (1 µmol H_2O_2/min) activity of gills and muscles of *C. gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

When the mean values of the groups along the column were compared, there were no significant differences ($p > 0.05$) among the groups before and after treatments. Although the MPO activity in the gills of the Ich infested fish was highest in the group D treated with 1500mg/L of the extracts in comparison with other extracts treated groups. Moreover, the normal control group A had the lowest MPO activity in their gills at the end of the dip treatments. The decrease was not however, statistically significant ($p >0.05$) (Table 66).

However, there were no significant differences ($p > 0.05$) in the MPO activities in the muscles among the ich-infested groups, before and after treatment. The MPO activity in the muscle of the Ich infested fish was highest in the group G treated with 4500mg/L of the extracts in comparison with other extracts treated groups. Meanwhile, the normal control (group A) had the lowest MPO activity in the gill at the end of the dip treatments. The decrease was not significantly different ($p > 0.05$) (Table 66).

Table 66: Changes in the myeloperoxidase (1 µmol H_2O_2/min) of Gills and Muscles of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental. group	Tissue	Before Treatment	After Treatment
A	Gill	0.06 ± 0.16^{a1}	0.07 ± 0.03^{a1}
	Muscle	0.08 ± 0.01^{a1}	0.06 ± 0.006^{a1}
B	Gill	0.38 ± 0.47^{ab1}	0.37 ± 0.46^{a1}
	Muscle	0.25 ± 0.33^{a1}	0.63 ± 0.47^{a1}
C	Gill	0.60 ± 0.35^{b1}	0.43 ± 0.60^{a1}
	Muscle	0.06 ± 0.02^{a1}	0.08 ± 0.012^{a1}
D	Gill	0.29 ± 0.180^{ab1}	1.06 ± 0.95^{a1}
	Muscle	0.05 ± 0.01^{a1}	0.21 ± 0.16^{a1}
E	Gill	0.09 ± 0.03^{a1}	0.94 ± 0.75^{a1}
	Muscle	0.06 ± 0.004^{a1}	0.48 ± 0.34^{a1}
F	Gill	0.15 ± 0.06^{ab1}	0.72 ± 0.56^{a1}
	Muscle	0.05 ± 0.02^{a1}	0.54 ± 0.55^{a1}
G	Gill	0.09 ± 0.007^{a1}	0.88 ± 0.70^{a1}
	Muscle	0.06 ± 0.02^{a1}	0.70 ± 0.55^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E(2500mg/L), F(3500mg/L) and G (4500mg/L).

3.19.2 Changes in the myeloperoxidase (1 µmol H_2O_2/min) activities in the gills and muscle of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 67 shows that the mean values of the groups along the column, showed no significant differences ($p > 0.05$) among the groups before treatment and in the 24h treatment, whereas from the 48h to 96h treatments, the MPO levels in the gills of the Ich-infested *C. gariepinus* among the groups differed significantly ($p < 0.05$). At the same time, the MPO levels in the extracts treated groups had marked significant decreases when compared to their baseline mean values. Similarly, the MPO levels in the negative control were equally reduced when compared to the positive control and the extracts groups which had higher MPO levels at the end of the short term treatments.

Furthermore, when the mean values of the groups along the column were compared, there were no significant differences ($p > 0.05$) in the MPO activities in the muscle among the groups, before treatment in the 24h and 72h post treatments. Moreover, significant differences ($p < 0.05$) were recorded in 48h and 96h only among the groups as shown in Table 67.

Table 67: Changes in myeloperoxidase (1 µmol H_2O_2/min) activities in the gills and Muscles of *C. gariepinus* after short treatments.

Experimental group	Tissue	Before Treatment	24h	48h	72h	96h
A	Gill	0.92 ± 0.69^{a1}	0.07 ± 0.09^{a2}	0.04 ± 0.009^{a2}	$0.01 \pm 0.03^{a2,3}$	0.067 ± 0.08^{a2}
	Muscle	0.074 ± 0.011^{a1}	0.16 ± 0.02^{a2}	0.07 ± 0.005^{ab1}	0.07 ± 0.005^{a1}	0.07 ± 0.003^{a2}
B	Gill	0.55 ± 0.40^{a1}	0.085 ± 0.04^{ab2}	0.06 ± 0.008^{b2}	0.03 ± 0.01^{a2}	0.06 ± 0.04^{a2}
	Muscle	0.60 ± 0.45^{a1}	0.10 ± 0.01^{a1}	0.08 ± 0.005^{bc2}	0.08 ± 0.003^{a2}	0.08 ± 0.02^{a2}
C	Gill	0.33 ± 0.47^{a1}	0.124 ± 0.05^{b1}	0.07 ± 0.002^{c2}	0.07 ± 0.009^{c2}	0.08 ± 0.007^{a2}
	Muscle	0.55 ± 0.42^{a1}	0.08 ± 0.01^{a2}	0.9 ± 0.003^{c1}	0.27 ± 0.26^{b1}	0.55 ± 0.39^{ab1}
D	Gill	1.23 ± 1.10^{a1}	0.010 ± 0.04^{ab2}	0.07 ± 0.004^{bc2}	0.08 ± 0.01^{a2}	0.08 ± 0.003^{a2}
	Muscle	0.34 ± 0.41^{a1}	0.08 ± 0.008^{a2}	0.09 ± 0.02^{c2}	0.07 ± 0.107^{a2}	0.07 ± 0.00^{52}
E	Gill	1.06 ± 1.04^{a1}	0.07 ± 0.009^{a2}	0.08 ± 0.001^{c2}	0.07 ± 0.009^{a2}	0.08 ± 0.004^{a2}
	Muscle	0.34 ± 0.43^{a1}	0.08 ± 0.007^{a2}	0.05 ± 0.01^{a2}	0.06 ± 0.01^{a2}	0.08 ± 0.007^{a2}
F	Gill	0.11 ± 0.89^{a1}	0.08 ± 0.008^{ab2}	0.08 ± 0.01^{c2}	0.07 ± 0.007^{a2}	0.08 ± 0.009^{a2}
	Muscle	0.11 ± 0.03^{a1}	0.34 ± 044^{a1}	0.08 ± 0.009^{bc2}	0.07 ± 0.01^{a2}	0.09 ± 0.006^{a2}
G	Gill	1.05 ± 1.05^{a1}	0.07 ± 0.007^{ab2}	0.08 ± 0.009^{c2}	0.85 ± 0.01^{a2}	0.77 ± 0.60^{b2}
	Muscle	0.41 ± 0.46^{a1}	0.08 ± 00.0^{a2}	0.08 ± 0.007^{bc2}	0.069 ± 0.117^{a2}	1.11 ± 0.99^{b1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

3.19.3. Changes in the MPO activity in the gills and muscles of *C. gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 68 shows that the mean value of the MPO in the gills of Ich infested fish were comparable to those in the control group (normal control) before the treatment commenced. Meanwhile, the MPO activities in the gills were not statistically significant ($p > 0.05$) when compared to the control along the column. In days 7 and 15, the MPO activities in the gills differed significantly ($p < 0.05$) among the groups.

Moreover, the MPO activity in the muscles of ich infested *C. gariepinus* was not comparable to the normal control in days 7 and day 15. There were significant differences ($p < 0.05$) among the groups throughout the prolonged bath treatments when the mean values along the column were compared. There was an increased level of MPO activity in each group when compared to their baselines (before treatment). The increase appeared to be steady and time-dependent except in group G treated with 45mg/L of the extracts which had mixed trends in MPO levels in the gills of the infested fish. Thereafter, the MPO activity in the muscles among the treated groups increased in a time-dependent manner when compared to the negative control that maintained a steady level of MPO in the muscles with reduced MPO activities in the gills of the infested fish as shown in Table 68.

Table 68: Changes in the activities of myeloperoxidase (1 µmol H_2O_2/min) in the gills and muscles of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental. group	Tissue	Before treatment	Day 7	Day 15
A	Gill	0.48 ± 0.58^{a1}	0.07 ± 0.01^{a2}	0.07 ± 0.01^{a2}
	Muscle	0.06 ± 0.01^{a1}	0.04 ± 0.01^{a1}	0.92 ± 0.01^{a2}
B	Gill	0.13 ± 0.09^{a1}	0.09 ± 0.07^{a1}	0.06 ± 0.006^{a1}
	Muscle	0.08 ± 0.007^{a1}	0.07 ± 0.006^{a1}	0.08 ± 0.004^{a1}
C	Gill	0.80 ± 0.62^{a1}	0.09 ± 0.07^{a1}	1.36 ± 1.58^{a2}
	Muscle	0.05 ± 0.01^{a1}	1.11 ± 0.03^{b2}	0.77 ± 0.51^{a2}
D	Gill	0.86 ± 0.007^{a1}	0.79 ± 0.44^{b1}	1.34 ± 0.75^{a1}
	Muscle	0.07 ± 0.03^{a1}	1.75 ± 0.71^{bc2}	3.09 ± 1.04^{b2}
E	Gill	0.15 ± 0.14^{a1}	0.88 ± 0.18^{b1}	0.82 ± 0.61^{a1}
	Muscle	0.07 ± 0.01^{a1}	2.86 ± 0.65^{d2}	3.22 ± 1.14^{b2}
F	Gill	0.18 ± 0.19^{a1}	0.79 ± 0.61^{b1}	1.50 ± 0.74^{a2}
	Muscle	0.04 ± 0.07^{a1}	2.56 ± 0.95^{cd2}	3.45 ± 1.46^{b2}
G	Gill	0.73 ± 0.53^{a1}	1.80 ± 0.41^{b1}	0.64 ± 0.67^{a1}
	Muscle	0.65 ± 0.02^{a1}	1.32 ± 0.43^{b1}	3.38 ± 1.40^{b1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L)

3.20. Changes in the Biochemical Parametrs of *Clarias gariepinus* Infested with *Ichthyophthirius multifilis* and Treated with Aqueous Extractss of *M. oleifera* Leaves and Standard Drug, before and after Dip, Short Term and Prolonged Bath Treatments

3.20.1 Changes in the total protein (g/dl) of *Clarias gariepinus* after dip treatments with aqueous extractss of *M. oleifera* leaves and standard drug (fish cure)

Table 69 Shows the changes in total protein levels of Ich infested *C. gariepinus* during dip treatments. It was observed that before treatment, the total protein levels among the Ich infested groups were significantly ($p < 0.05$) high when compared to the normal control, while comparing means along the column. Although after the dip (1 h) treatment, no significant ($p > 0.05$) changes were recorded among the treated groups and the normal control. While comparing the treated groups with the negative control, the TP levels in the negative control was higher in comparison with the extractss and standard drug treated groups. Among the treated groups, while comparing means along the colunm, the total protein levels appeared reduced among each groups at each duration of exposure.

Table 67: Changes in the total protein (g/dl) levels of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	After Treatment
A	2.71 ± 0.008^{a1}	2.35 ± 0.37^{a1}
B	3.84 ± 0.78^{ab1}	3.64 ± 0.06^{a1}
C	3.24 ± 0.57^{ab1}	3.08 ± 0.85^{a1}
D	2.88 ± 0.46^{a1}	2.77 ± 0.96^{a1}
E	4.10 ± 0.1^{b1}	3.60 ± 0.78^{a1}
F	3.62 ± 1.06^{ab1}	2.81 ± 0.89^{a1}
G	4.29 ± 0.57^{b1}	3.59 ± 0.21^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L)

3.20.2. Changes in the total protein (g/dl) levels of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 70 shows the changes in the total protein levels of Ich infested *C. gariepinus* during dip treatments. It was observed that before treatment, the total protein levels among the Ich infested groups were not significantly different ($p > 0.05$) when compared to the normal control (group A) along the column. During the short term treatment in 24h and 72h, no significant difference ($p > 0.05$) were recorded among the groups, although in 24h, the total protein level in the normal control was reduced compared to other groups (B-G). However, in the 72h treatment, the total protein level in the negative control was reduced when compared to the treated groups and the normal control. Moreover, during the 48h and 96h treatments, the total protein levels among the Ich infested groups differed significantly when compared to the normal control. The significant changes in the total protein levels among the Ich infested groups were not concentration dependent. There was a significant decrease in the total protein of the treated groups when compared to their baselines and in each of the treatment intervals along the column. Moreso, in the negative control, the TP level was highest in the 96 h when compared to other treated groups that had reduced TP levels at the end of the exposure period.

Table 70: Changes in the total protein (g/dl) levels of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	24h	48h	72h	96h
A	2.75 ± 0.59^{a1}	1.36 ± 16^{a1}	2.02 ± 1.16^{a}	12.81 ± 0.81^{b1}	2.44 ± 0.60^{a1}
B	4.00 ± 0.90^{ab1}	2.17 ± 0.05^{ab1}	2.71 ± 0.44^{a1}	1.64 ± 0.83^{a1}	4.95 ± 1.94^{b1}
C	3.72 ± 0.44^{ab1}	3.79 ± 1.60^{b1}	5.00 ± 1.79^{b1}	2.86 ± 0.37^{b1}	3.09 ± 0.94^{a1}
D	3.34 ± 0.95^{ab1}	3.06 ± 123^{ab1}	2.28 ± 0.27^{a1}	2.56 ± 0.13^{b1}	2.89 ± 0.018^{a1}
E	4.01 ± 6.40^{ab1}	2.92 ± 0.43^{ab1}	2.61 ± 0.56^{a1}	2.38 ± 0.13^{ab1}	2.52 ± 0.59^{a1}
F	4.06 ± 0.56^{b1}	3.82 ± 1.89^{b1}	2.27 ± 0.21^{a1}	2.44 ± 0.11^{ab1}	2.36 ± 0.42^{a1}
G	3.21 ± 0.53^{ab1}	2.24 ± 0.86^{ab1}	2.95 ± 0.91^{a1}	2.28 ± 0.04^{ab1}	1.47 ± 098^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L)

3.20.3. Changes in the total protein levels (g/dl) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 71 Shows the changes in the total protein levels of Ich infested *C. gariepinus* during prolonged bath treatments. It was observed that before treatment, the total protein levels among the Ich infested groups were not significantly different ($p > 0.05$) when compared to the normal control (group A) along the column. However, during the prolonged bath treatments, at the end of day 7, the total protein levels among the treated and the negative control, differed significantly ($p<0.05$) when compared to the normal control. Meanwhile, at the end of the prolonged bath treatments, the total protein levels among the extracts treated groups were higher than the standard drug treated group (group C). Moreso, at the end of the prolonged bath treatments, the total protein levels did not differ significantly ($p > 0.05$) among the Ich infested groups and the normal control. But the total protein levels in the normal control were fairly reduced in day 15 when compared to the other groups and the negative control that had an elevated TP level. The significant change in the total protein levels among the Ich infested groups along the column was not concentration-dependent. There were significant increases ($p < 0.05$) in total protein levels among the treated groups when compared to their baselines (before treatment) at the end of the prolonged bath treatments. Moreso, in the negative control, the increased TP were lower when compared to the treated groups and higher when compared to the normal control. Although the normal control had a reduced total protein throughout the prolonged bath treatment (day 7 and day 15) in comparison with other Ich-infested groups.

Table 71: Changes in the total protein levels (g/dl) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	Day 7	Day 15
A	4.81 ± 0.97^{b1}	2.83 ± 0.57^{a1}	4.16 ± 0.2^{a1}
B	3.35 ± 0.41^{a1}	3.66 ± 0.43^{a1}	$5.77 \pm 2.09ab^{ab1}$
C	3.05 ± 0.48^{a1}	4.17 ± 1.05^{ab1}	5.89 ± 1.35^{ab1}
D	3.46 ± 0.62^{a1}	5.54 ± 1.57^{abc1}	7.31 ± 0.96^{b1}
E	4.24 ± 0.78^{ab1}	8.02 ± 2.25^{c1}	7.50 ± 2.17^{b1}
F	3.43 ± 0.94^{a1}	5.84 ± 2.07^{abc1}	7.36 ± 098^{b1}
G	3.40 ± 0.30^{a1}	6.96 ± 2.11^{bc1}	7.08 ± 1.67^{b1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infected not treated), C (infected treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L)

3.20.4. Changes in the albumin levels (g/dl) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 72 shows the changes in the albumin levels of Ich-infested *C. gariepinus* during short term treatments. It was observed that before treatment, the albumin levels among the Ich infested treated and untreated groups were not statistically significant ($p > 0.05$) compared to the healthy control (group A) while comparing means along the column. Although after the dip (1h) treatments, no significant ($p > 0.05$) changes were recorded among the treated groups and the healthy control.

It was observed that the albumin levels appeared elevated especially in groups C, D, F, and G except the group E that had fairly reduced albumin level at the end of the dip treatments. Increased level in the albumin level was time-dependent. However, the albumin levels among the treated groups were higher than the albumin level in the negative control at the end of the dips treatment.

Table 72: Changes in albumin levels (g/dl) of *C. gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before Treatment	After Treatment
A	2.33 ± 0.08^{b1}	1.89 ± 0.42^{a1}
B	1.30 ± 1.5^{ab1}	1.76 ± 1.05^{a1}
C	1.72 ± 0.01^{ab1}	1.80 ± 0.99^{a1}
D	1.87 ± 0.55^{ab1}	2.10 ± 0.06^{a1}
E	1.81 ± 0.59^{ab1}	1.77 ± 0.50^{a1}
F	1.03 ± 0.78^{a1}	2.19 ± 0.09^{a1}
G	1.19 ± 0.23^{ab1}	1.79 ± 0.49^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F(3500mg/L) and G (4500mg/L)

3.20.5. Changes in the albumin levels of *Clarias gariepinus* after short-term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 73 shows the changes in the albumin levels of Ich-infested *C. gariepinus* during short term treatments. It was observed that before treatment, the albumin levels among the Ich-infested groups were not significantly different ($p > 0.05$) when compared to the healthy control along the column. In the 24h treatment, significantly ($p < 0.05$) high albumin levels were recorded among the groups. Meanwhile, the albumin levels in the untreated group were lower compared to the treated groups and the healthy control. But between the 48h, 72h and 96h treatments, no significant ($p > 0.05$) changes were observed among the treated, untreated and healthy control. But it was observed that the albumin levels in the untreated groups remained reduced when compared with other groups in the same column.

Table 73: Changes in the albumin levels (g/dl) of *Clarias gariepinus* after short term treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before treatment	24h	48h	72h	96h
A	1.53 ± 0.38^{a1}	1.20 ± 0.08^{ab1}	1.44 ± 1.23^{a1}	1.47 ± 0.55^{a1}	2.13 ± 0.81^{a1}
B	0.72 ± 0.01^{a1}	0.61 ± 0.700^{a1}	1.32 ± 0.96^{a1}	1.33 ± 0.75^{a1}	1.45 ± 1.37^{a1}
C	1.13 ± 1.00^{a1}	1.60 ± 0.47^{bcd1}	2.52 ± 0.54^{a1}	1.33 ± 0.17^{a1}	2.19 ± 0.50^{a1}
D	1.10 ± 0.96^{a1}	2.01 ± 0.25^{cd1}	2.23 ± 0.59^{a1}	1.17 ± 0.98^{a1}	2.02 ± 6.70^{a1}
E	1.04 ± 1.23^{a1}	1.99 ± 0.17^{cd1}	1.96 ± 0.13^{a1}	1.95 ± 0.36^{a1}	1.53 ± 0.83^{a1}
F	0.58 ± 0.53^{a1}	2.06 ± 0.07^{d1}	1.49 ± 1.14^{a1}	2.08 ± 0.36^{a1}	2.32 ± 0.33^{a1}
G	0.87 ± 0.49^{a1}	1.36 ± 0.19^{bc1}	1.76 ± 0.60^{a1}	2.22 ± 0.12^{a1}	1.19 ± 0.83^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F(350mg/L) and G (450mg/L)

3.20.6 Changes in the albumin levels of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 74 Shows the changes in the albumin levels of Ich-infested *C. gariepinus* during prolonged bath treatments. It was observed that before treatment, the albumin levels among the Ich infested groups were significant ($p < 0.05$), along the column. However, during and after the prolonged bath treatments, the albumin levels among the Ich-infested treated groups and the healthy control did not differ significantly ($p > 0.05$).

Although, by comparing their albumin levels, the albumin level of the healthy control were lower compared to the treated and non treated groups. However, at the end of the prolonged bath treatments. There were fairly increased albumin levels among the extracts treated groups and the standard control between their baselines (pre treatment) and the post treatment (day 15).

Table 74: Changes in the albumin levels (g/dl) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before Treatment	Day 7	Day 15
A	1.12 ± 0.19^{bc1}	1.10 ± 0.35^{a1}	1.25 ± 0.19^{a1}
B	0.92 ± 0.34^{ab1}	1.44 ± 0.64^{a1}	2.03 ± 0.90^{a1}
C	1.72 ± 0.52^{c1}	1.44 ± 0.61^{a1}	1.79 ± 0.34^{a1}
D	1.02 ± 0.19^{ab1}	1.86 ± 0.65^{ab1}	2.11 ± 0.0041^{a}
E	1.07 ± 0.12^{abc1}	2.56 ± 0.74^{ab1}	1.72 ± 0.51^{a1}
F	0.46 ± 0.23^{a1}	3.22 ± 1.41^{b2}	2.06 ± 0.11^{a2}
G	1.15 ± 0.52^{bc1}	2.54 ± 0.75^{ab1}	1.74 ± 0.56^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L)

3.20.7. Changes in the globulin (g/dl) levels of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 75 shows the changes in the globulin levels of *C. gariepinus* after dip treatments. During the dip treatments the globulin levels in the Ich-infested groups before treatment were not significant ($p > 0.05$) when compared to the healthy control, although the healthy control had a reduced globulin level. At the same times, no significant differences ($p > 0.05$) were recorded in the globulin levels of the treated and untreated groups when compared to the control.

Table 75: Changes in globulin levels (g/dl) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before Treatment	After Treatment
A	0.53 ± 0.28^{a1}	0.60 ± 0.17^{ab1}
B	1.87 ± 1.45^{a1}	1.60 ± 1.29^{ab1}
C	1.17 ± 0.45^{a1}	0.92 ± 0.48^{ab1}
D	0.98 ± 0.87^{a1}	0.21 ± 0.22^{a1}
E	1.90 ± 1.00^{a1}	1.83 ± 0.69^{ab1}
F	1.43 ± 0.52^{a1}	0.61 ± 0.85^{ab1}
G	1.59 ± 0.91^{a1}	1.80 ± 0.71^{b1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), (3500mg/L) and G (4500mg/L)

3.20.8. Changes in the globulin (g/dl) levels of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 76 Shows the changes in the globulin levels of *C. gariepinus* after short term treatments. The globulin levels in the Ich infested groups be fore treatment, were not significantly different ($p > 0.05$) compared to the healthy control during the short term treatment. The globulin levels did not differ significantly ($p > 0.05$) in the 24h, 48h and 72h treatments although they differed significantly ($p < 0.05$) at the end of the 96h treatment.

The globulin levels were significantly reduced when compared with the means of the groups at the end of the treatment with their globulin baseline values. Meanwhile, the negative control had elevated globulin levels at the end of the short term treatment (96 h) in contrast to the treated groups and healthy control that had reduced globulin levels. The globulin levels decreased in the treated groups in a duration dependent manner.

Table 76: Changes in globulin levels (g/dl) of *C. gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	24h	48h	72h	96h
A	0.71 ±0.08^{a1}	0.35 ±0.43^{a1}	0.54 ±0.15^{a1}	1.41 ±1.03^{a1}	0.61 ± 0.07^{a1}
B	1.45 ± 0.76^{a1}	1.56 ±0.66^{a1}	1.42 ±0.95^{a1}	0.55 ± 0.21^{a1}	1.87 ± 1.06^{b1}
C	1.6 ± 1.18^{a1}	1.25 ±0-63^{a1}	1.62 ±1.47^{a1}	1.38 ±0.57^{a1}	0.73 ± 0.01^{a1}
D	1.21 ± 1.00^{a1}	0.99 ±1.01^{a1}	0.43 ±0.31^{a1}	1.51 ± 0.95^{a1}	0.61 ± 0.34^{a1}
E	1.80 ± 0.89^{a1}	1.00 ±0.22^{a1}	0.45 ±0.38^{a1}	0.56 ± 0.25^{a1}	0.78 ± 0.05^{a1}
F	1.70 ±0.70^{a1}	0.71 ±0.52^{a1}	1.58 ±0.69^{a1}	0.57 ± 0.30^{a1}	0.76 ± 0.02^{a1}
G	1.81 ±0.83^{a1}	1.07 ±0.89^{a1}	1.35 ±0.79^{a1}	0.32 ± 0.38^{a1}	0.07 ± 0.03^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different numbers as superscript in a row are significantly different (P<0.05).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L)

3.20.9. Changes in the globulin levels of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 77 shows the changes in the globulin levels of Ich-infested *C. gariepinus* during prolonged bath treatments. It was observed that before treatment, the globulin levels among the Ich infested groups did not differ significantly ($p > 0.05$) when compared to the healthy control along the column. However, during the prolonged bath treatments at the end of day 7 and day 15, the globulin levels differed significantly ($p < 0.05$) among the treated and untreated groups when compared to the healthy control. Significant ($p < 0.05$) high globulin levels were recorded among the treated groups at each duration of exposure but at the end of the prolonged bath treatments, globulin levels among the treated groups were higher compared to their baselines (before treatment).

Table 77: Changes in globulin levels (g/dl) of *C. gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	Day 7	Day 15
A	0.76 ± 0.18^{a1}	1.03 ± 0.18^{a1}	2.91 ± 0.42^{abc1}
B	1.15 ± 0.99^{a1}	2.18 ± 0.45^{ab1}	1.41 ± 0.89^{a1}
C	1.45 ± 0.70^{a1}	2.73 ± 1.60^{abc1}	3.99 ± 1.57^{bc1}
D	0.87 ± 0.58^{a1}	4.35 ± 1.51^{bc2}	4.25 ± 0.78^{c2}
E	0.80 ± 0.64^{a1}	3.32 ± 1.46^{bc2}	2.05 ± 1.11^{ab2}
F	1.58 ± 0.70^{a1}	4.80 ± 1.05^{c1}	2.38 ± 1.50^{abc1}
G	1.22 ± 0.79^{a1}	4.41 ± 1.36^{bc1}	3.73 ± 0.55^{bc1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L)

3.20.9. Changes in the Urea levels (mg/dL) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 78 shows the changes in the urea levels of Ich-infested *C. gariepinus* during dip treatments. It was observed that before treatment, the urea levels among the parasitized groups were significantly ($p < 0.05$) higher compared to the healthy control. But at the end of the dip treatments the urea levels among the treated groups when compared to the negative control, did not differ significantly ($p > 0.05$), although the urea levels in the untreated groups were higher compared to the treated groups and the healthy controls. The urea levels decreased in each duration among the treated groups in a time-dependent manner. Meanwhile, both the negative and healthy controls had elevated urea levels, but the urea level in the healthy control was lower than the negative control and the treated groups.

Table 78: Changes in the urea levels (mg/dL) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before Treatment	After treatment
A	16.24 ± 3.70^{a1}	19.56 ± 0.67^{a1}
B	24.41 ± 3.08^{bc1}	27.60 ± 11.65^{a1}
C	21.33 ± 2.95^{ab1}	19.76 ± 4.45^{a1}
D	20.89 ± 2.51^{ab1}	19.95 ± 0.25^{a1}
E	25.81 ± 3.84^{bc1}	20.47 ± 1.69^{a1}
F	28.33 ± 3.69^{c1}	24.65 ± 1.58^{a1}
G	83.77 ± 4.42^{bc1}	22.57 ± 1.30^{a2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F(3500mg/L) and G (4500mg/L)

3.20.10. Changes in the urea levels of *Clarias gariepinus* after short-term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Table 79 shows the changes in the urea levels of *C. gariepinus* during short term treatments. It was observed that before treatment, the urea levels in the Ich-infested groups, differed significantly ($p < 0.05$) when compared to the healthy control. However, during the short term treatments, in 24h, the urea levels among the treated groups did not differ significantly ($P > 0.05$) when compared to the healthy control. The healthy control had a decreased urea level when compared to the treated groups and the negative control. In the 48h and 96h treatments, the urea levels among the groups differed significantly ($p < 0.05$) except in the 72h treatment were no significant changes were observed ($p > 0.05$). The negative control had a peak urea level in the 48h, although throughout the treatment periods, the urea levels were significantly higher compared to other groups. The urea levels among the extracts treated group (D - G) were increased in each duration of exposure especially in 96 h except in group C treated with standard drug which had reduced urea levels. The healthy control maintained a decreased urea level over time compared to the treated and untreated groups.

Table 79: Changes in urea levels (mg/dL) of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	24 h	48 h	72 h	96 h
A	24.59 ± 1.44^{a1}	19.89 ± 3.10^{a2}	30.10 ± 9.95^{a1}	38.93 ± 6.75^{a1}	24.70 ± 3.80^{a1}
B	33.05 ± 55.76^{bc1}	38.22 ± 13.38^{b1}	68.54 ± 12.76^{b2}	65.98 ± 12.04^{b2}	54.68 ± 15.96^{c2}
C	36.03 ± 3.37^{c1}	27.62 ± 8.04^{ab1}	36.21 ± 16.56^{a1}	49.24 ± 18.68^{ab2}	32.58 ± 5.01^{ab1}
D	27.32 ± 2.26^{ab1}	24.22 ± 6.15^{a1}	39.01 ± 13.47^{a1}	46.01 ± 10.30^{ab2}	39.39 ± 9.39^{abc1}
E	28.31 ± 5.94^{ab1}	21.22 ± 2.04^{a1}	37.84 ± 3.34^{a1}	36.15 ± 4.41^{a1}	39.97 ± 1.01^{ab1}
F	28.61 ± 1.95^{ab1}	32.37 ± 4.86^{ab1}	31.94 ± 10.11^{a1}	41.50 ± 5.87^{a2}	44.99 ± 7.12^{bc2}
G	31.71 ± 2.30^{ab1}	28.78 ± 3.98^{ab1}	$44.63 \pm 5.50^{a2,1}$	43.48 ± 15.07^{a2}	34.31 ± 8.78^{ab2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).. A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F(350mg/L) and G (450mg/L)

3.20.11. Changes in the urea level of *Clarias gariepinus* after prolonged bath treatments with aqueous extractss of *Moringa oleifera* leaves and standard drug (fish cure).

Table 78 shows the changes in the urea level of Ich-infested *C. gariepinus* after prolonged bath treatments. The urea levels among the parasitized groups were not significantly different ($p > 0.05$) when compared to the healthy control (group A). During the prolonged bath treatments at the end of day 7, the urea levels among the treated groups differed significantly ($p < 0.05$), although the urea level in group C (standard drug) were highly decreased in comparison to the extracts treated groups and the control (healthy and negative). At the end of the prolonged bath treatments, the urea levels did not differ significantly ($p > 0.05$) among the treated groups and the negative control, although the urea levels in the healthy control were highly decreased in comparison to other groups.

Moreso, the groups treated with varied concentrations of the extracts had increased urea levels at the end of the treatments when compared to their baselines. At the same time, the positive control had decreased urea levels though the decrease did not differ significantly ($p > 0.05$) when compared to the other treated groups. The healthy control maintained a reduced urea levels throughout the prolonged bath treatments (day 7 and 15).

Table 80: Changes in urea levels (mg/dL) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	Day 7	Day 15
A	19.84 ± 1.30^{a1}	20.56 ± 1.58^{a1}	$16.93. \pm 1.65^{a1}$
B	29.39 ± 2.10^{ab1}	34.50 ± 4.09^{b1}	34.38 ± 3.68^{b1}
C	32.41 ± 2.68^{b1}	19.96 ± 2.30^{a2}	28.33 ± 8.27^{ab1}
D	22.20 ± 4.42^{ab1}	32.98 ± 4.63^{b1}	32.94 ± 14.53^{b1}
E	28.57 ± 4.02^{ab1}	29.38 ± 10.57^{ab1}	35.68 ± 5.50^{b1}
F	25.50 ± 8.57^{ab1}	28.30 ± 2.04^{ab1}	34.91 ± 4.69^{b1}
G	28.91 ± 9.48^{ab1}	36.17 ± 4.36^{b1}	32.64 ± 4.87^{b1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L)

3.20.11. Changes in the creatinine (mg/dl) level of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 81 shows the changes in the creatinine levels of *C. gariepinus* during dip treatments. It was observed that before treatment, the creatinine levels among the parasitized groups were not significantly different ($p > 0.05$) compared to the healthy control. After the dip treatment, no significant changes were observed among the treated groups and the healthy control, though the negative control had elevated creatinine level compared to the treated groups.

The creatinine levels showed significant ($p < 0.05$) increases in each duration among some extracts treated groups (D, F and G) while the group F had a reduced creatinine level. At the same time, the group treated with standard drug had elevated creatinine level throughout the exposure period. Thereafter, the negative control had the highest creatinine level in comparison to other treated groups before and after treatments.

Table 81: Changes in the creatinine levels (mg/dL) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before treatment	After treatment
A	1.48 ± 0.6^{ab1}	1.65 ± 0.29^{a1}
B	1.71 ± 0.69^{b1}	2.19 ± 1.00^{a1}
C	0.85 ± 0.43^{ab1}	1.19 ± 0.35^{a1}
D	0.78 ± 0.14^{a1}	1.62 ± 0.07^{a1}
E	1.02 ± 0.33^{ab1}	1.63 ± 0.33^{a1}
F	1.30 ± 0.49^{ab1}	1.22 ± 0.96^{a1}
G	1.02 ± 0.33^{ab1}	1.37 ± 1.02^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L)

3.20.12. Changes in the creatinine levels of *C. gariepinus* infested with *I. multifillis* after short term treatment with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 82 shows the changes in the creatinine levels of *C. gariepinus* after short term treatment. It was observed that before treatment, the creatinine levels among the Ich infested groups were significantly higher ($p < 0.05$) when compared to the healthy control (group A). Meanwhile, throughout the short term treatments, no significant differences ($p > 0.05$) in the creatinine levels were observed among the groups along the column. The negative control had fairly elevated creatinine levels in each duration of treatments when compared with other groups throughout the short term treatment. The creatinine levels in each group, especially the treated groups had increased creatinine and at the dame time, the negative control also had the highest elevated creatinine level when compared to the healthy control in relation to the duration of exposure in a time-dependent manner.

Table 82: Changes in the creatinine levels (mg/dL) of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before treatment	24h	48h	72h	96h
A	0.59 ± 0.10^{b1}	0.17 ± 0.03^{a1}	0.28 ± 0.31^{a1}	0.36 ± 0.12^{a1}	0.66 ± 0.08^{a1}
B	0.20 ± 0.10^{a1}	0.43 ± 0.31^{a1}	1.84 ± 1.65^{b1}	1.31 ± 6.80^{b1}	0.97 ± 1.07^{a1}
C	0.31 ± 0.64^{a1}	0.37 ± 0.21^{a1}	0.37 ± 0.17^{a1}	0.69 ± 0.15^{a1}	0.69 ± 0.17^{a1}
D	0.17 ± 0.01^{a1}	0.31 ± 0.17^{a1}	0.23 ± 0.24^{a1}	0.59 ± 0.29^{a1}	0.49 ± 0.24^{a1}
E	0.25 ± 0.12^{a1}	0.38 ± 0.23^{a}	0.54 ± 0.21^{a1}	0.54 ± 0.04^{a1}	0.40 ± 0.21^{a1}
F	0.19 ± 0.10^{a1}	0.43 ± 0.19^{a1}	0.35 ± 0.22^{a1}	0.56 ± 0.19^{a1}	0.57 ± 0.02^{a1}
G	0.21 ± 0.14^{a1}	0.24 ± 0.28^{a1}	0.31 ± 0.24^{a1}	0.47 ± 0.05^{a1}	0.56 ± 0.22^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L)

3.20.13 Changes in the creatinine (mg/dl) levels of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 83 shows the changes in the creatinine levels of Ich infested *C. gariepinus* during prolonged bath treatments. Meanwhile, before treatment, the creatinine levels among the Ich infested groups were not significantly different ($p > 0.05$). Whereas throughout the prolonged bath treatments, the creatinine levels among the groups differed significantly ($p < 0.05$) when compared to the control. When the means along the column were compared, the creatinine levels in each group, especially the treated groups had elevated creatinine levels over-time when compared to the healthy control. In contrast, the creatinine levels in the negative control increased over time were not comparable with the treated groups that had significantly ($p<0.05$) higher creatinine levels at the end of the treatments when compared with their baselines.

Table 83: Changes in creatinine levels (mg/dL) of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before treatment	Day 7	Day 15
A	0.42 ± 0.09^{a1}	0.56 ± 0.24^{a1}	0.53 ± 0.07^{a1}
B	1.06 ± 0.39^{a1}	0.88 ± 0.26^{ab1}	1.21 ± 0.44^{c1}
C	1.17 ± 0.47^{a1}	1.65 ± 0.63^{bc1}	1.75 ± 0.16^{b1}
D	1.17 ± 0.87^{a1}	1.52 ± 0.49^{abc1}	1.77 ± 0.54^{bc1}
E	0.82 ± 0.61^{a1}	1.39 ± 0.61^{abc1}	1.95 ± 0.14^{bc1}
F	0.94 ± 0.41^{a1}	1.94 ± 0.68^{c1}	1.86 ± 0.0^{abc1}
G	0.49 ± 0.19^{a}	2.11 ± 0.77^{c1}	1.93 ± 0.20^{c}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05Mean values with different numbers as superscript in a row are significantly different (P<0.05).A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L)

3.20.14. Changes in the aspartate aminotransferase (I/U) levels of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 84 shows the changes in the AST levels of Ich-infested *C. gariepinus* during dip treatments. It was observed that before treatment, the AST levels among the Ich-infested groups were significantly ($p < 0.05$) low when compared to the healthy control, while comparing means along the columns were compared. Meanwhile, after the 1h treatments, no significant ($p > 0.05$) changes were seen in the AST levels of the treated and untreated groups when compared to the healthy control. The AST level in the negative control at the end of the 1h treatment was elevated compared to standard drug and group treated with extractss.The increase did not differ significantly with the healthy control and the treated groups.

It was further observed that the AST levels were fairly increased among the treated groups after treatments. However, in the negative control, the increased AST level was compared to other groups at the end of the 1 h treatments and the increase did not differ significantly ($p > 0.05$). There was also slight increase in the extracts treated groups (E, F and G) which did not differ significantly ($P>0.05$) when compared to the group D that had a lower AST levels, whereas, the group C treated and standard drug had slight increases in AST levels. The elevated AST levels in the treated and untreated group were time-dependent.

Table 82: Changes in the aspartate aminotransferase (I/U) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	After Treatment
A	31.00 ± 1.73^{b1}	33.33 ± 5.77^{a1}
B	16.33 ± 3.05^{a1}	43.33 ± 15.27^{a2}
C	28.67 ± 13.05^{b1}	38.66 ± 2.88^{a1}
D	23.66 ± 4.93^{ab1}	25.66 ± 6.02^{a1}
E	16.00 ± 1.73^{a1}	30.66 ± 8.32^{a2}
F	17.33 ± 0.57^{a1}	35.00 ± 8.66^{a2}
G	15.00 ± 2.00^{a1}	32.33 ± 4.61^{a2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L)

3.20.15. Changes in the aspartate aminotransferase (I/U) levels of *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 85 shows the changes in the AST levels of Ich infested *C. gariepinus* during short-term treatments. It was observed that before treatment, the AST levels among the Ich-infested treated groups were not significantly different ($p > 0.05$) when compared to the normal control (group A) along the column. The AST levels in the healthy control group were lower when compared to the parasitized groups before treatment. During the short term treatments in the 24h, 48h and 72h, the AST levels among the treated and untreated groups varied significantly ($p < 0.05$) but did not differ significantly ($P>0.05$) among the treated groups in the 96 h and their baselines (pre treatment). Meanwhile, the untreated group (negative control) maintained a peak level of AST enzyme activity in the serum of the infested fish and the rapid increase was higher when compared to other treated groups and normal control in each duration of exposure.

Table 85: Changes in aspartate aminotransferase (I/U) levels (I/U) of *Clarias gariepinus* in short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before treatment	24 h	48 h	72 h	96 h
A	54.00 ±28.68^{a1}	50.00 ±21.79 ^{a1}	55.00 ±30.41^{a1}	45.00 ±13.22^{a1}	46.67 ±11.54^{a1}
B	68.66 ±25.48^{a1}	31.67 ±5.77 ^{a1}	146.33 ±28.72^{b2}	91.67 ±15.27^{ab1}	115.00 ±47.69^{a2}
C.	98.00 ±20.12^{a1}	78.33 ± 20.21^{b1}	55.00 ± 8.66 ^{a1}	50.00 ± 21.79^{a1}	54.33 ± 17.92^{a1}
D	84.33 ± 27.32^{a1}	36.67 ± 12.58 ^{a2}	84.00 ± 18.24^{a1}	69.66 ± 4.04^{ab1}	74.00 ± 6.00^{a1}
E	81.33 ± 17.61^{a1}	28.33 ± 5.77 ^{a2}	91.66 ± 15.27^{a1}	65.00 ± 36.05^{ab1}	74.33 ± 4.04^{a1}
F	84.66 ± 9.23^{a1}	36.67 ± 12.58^{a2}	68.33 ± 20.81^{a1}	63.33 ± 36.85^{ab1}	64.33 ± 11.15^{a1}
G	82.00 ± 18.52^{a1}	31.67 ± 11.54 ^{a2}	93.33 ± 40.10^{a1}	58.33 ± 14.43^{ab1}	64.00 ± 6.00^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different numbers as superscript in a row are significantly different (P<0.05). A (control), B (infected not treated), C (infected treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L)

3.20.16. Changes in the aspartate aminotransferase (I/U) levels of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 86 shows the changes in the AST levels of Ich-infested *C. gariepinus* during prolonged bath treatments. It was observed that before treatment, the AST levels among the Ich infested groups were not significantly different ($p > 0.05$) when compared to the normal control (group A) along the column. However, during the prolonged bath treatment in day 7, there was a significant difference ($p < 0.05$) in the AST levels among the treated and the negative control when compared to the healthy control, whereas, at the end of the prolonged bath treatments, the AST levels among the extracts treated groups were fairly higher than the standard drug treated group. Furthermore, when the treated groups (standard drug and extracts treated groups) were compared with the negative control, the AST levels were fairly reduced compared to the negative control. Moreover, the healthy control maintained a reduced AST level throughout the exposure period when compared to other groups that had fairly elevated AST level.

Table 86: Changes in aspartate aminotransferase (I/U) levels in *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	Day 7	Day 15
A	74.66 ± 10.01^{a1}	50.00 ± 5.10^{a1}	68.33 ± 2.88^{a1}
B	87.67 ± 5.85^{a1}	70.10 ± 5.10^{b1}	106.66 ± 25.65^{b1}
C	84.33 ± 4.61^{a1}	90.10 ± 13.22^{c1}	90.00 ± 8.66^{ab1}
D	$76.33 \pm 1\text{-}.26^{a1}$	91.67 ± 2.88^{c1}	100.00 ± 5.10^{b1}
E	89.00 ± 6.55^{a1}	88.67 ± 6.35^{c1}	98.33 ± 2.88^{b1}
F	85.00 ± 13.11^{a1}	90.00 ± 8.66c1	98.33 ± 11.54^{b1}
G	82.00 ± 2.65^{a1}	86.00 ± 5.00^{bc1}	101.76 ± 2081^{b1}

Mean values with the same alphabets as superscript in a column are not significantly different (P>0.05). Mean values with different numbers as superscript in a row are significantly different (P<0.05).A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L)

3.20.17 Changes in the alanine aminotransferase (I/U) levels in *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 87 shows the changes in the ALT levels of Ich-infested *C. garipinus* during dip treatments. It was observed that before treatment, the ALT levels among the parasitized groups were not significantly different ($p > 0.05$) when compared to the healthy control, when the means were compared along the column. However, at the end of the dip treatments no significant ($p > 0.05$) changes were recorded among the treated groups (extracts and standard groups). When the treated groups were compared with the negative control, the ALT levels in the treated groups were highest in the negative control throughout the dip treatments compared to the negative control and the treated groups.

Table 87: Changes in alanine aminotransfarase levels (I/U) of *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before treatment	After Treatment
A	46.00 ± 13.52^{a1}	42.33 ± 17.50^{ab1}
B	54.00 ± 5.29^{ab1}	59.33 ± 1.15^{a1}
C	51.00 ± 7.54^{ab1}	45.66 ± 14.84^{ab1}
D	55.33 ± 7.57^{ab1}	49.66 ± 13.65^{ab1}
E	52.67 ± 3.05^{ab1}	43.33 ± 14.43^{ab1}
F	60.67 ± 1.15^{b1}	44.33 ± 10.69^{ab1}
G	5.33 ± 5.50^{ab1}	49.33 ± 7.50^{ab1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L)

3.20.18. Changes in the alanine aminotransferase levels (I/U) in *Clarias gariepinus* during short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 88 shows the changes in the ALT levels of Ich-infested *C. gariepinus* during short term treatments. It was observed that before treatment, the ALT levels among the Ich-infested groups were not significantly different ($p > 0.05$) when compared to the healthy control (Group A). During the short term treatments the 24h, no significant difference ($p > 0.05$) was recorded among the treated and untreated groups compared to the healthy control. Meanwhile, significant ($p < 0.05$) change in ALT levels were observed among the treated groups and the negative control, with higher ALT level recorded in the negative control in the 48h treatments. Moreover, in 72h and 96h treatments, the ALT levels among the treated groups differed significantly ($p < 0.05$) when compared to the healthy control. The healthy control had maintained reduced ALT levels when compared to the parasitized treated and untreated groups throughout the short-term treatments. At the end of the short-term treatment (96h), elevated ALT levels were observed among the treated groups and normal control when compared to the negative control. The increased ALT levels in the negative control were observed almost in each duration of exposure (24 ï 96 h) when compared among other groups.

Table 88: Changes in alanine aminotransferase (I/U) levels in *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	24 h	48 h	72 h	96 h
A	49.33 ± 7.02^{a1}	53.33 ± 31.75^{a1}	41.00 ± 6.55^{a1}	38.33 ± 14.01^{a1}	51.33 ± 1.15^{a1}
B	45.00 ± 13.22^{a1}	61.66 ± 10.40^{a1}	80.00 ± 22.91^{b1}	41.33 ± 7.02^{ab1}	65.66 ± 7.76^{b1}
C	53.00 ± 4.58^{a1}	$48.\ 33 \pm 2.88^{a1}$	50.00 ± 6.92^{a1}	56.00 ± 7.54^{c1}	54.33 ± 8.14^{a1}
D	54.00 ± 3.46^{a1}	56.67 ± 10.40^{a1}	49.67 ± 2.08^{a1}	50.66 ± 2.08^{abc1}	59.66 ± 2.5^{ab1}
E	51.00 ± 1.73^{a1}	56.66 ± 12.58^{a1}	43.33 ± 9.29^{a1}	55.00 ± 6.92^{bc1}	58.66 ± 2.30^{ab1}
F	53.66 ± 4.04^{a1}	66.66 ± 5.77^{a1}	41.10 ± 6.92^{a1}	53.66 ± 3.51^{bc1}	58.00 ± 3.46^{ab1}
G	57.66 ± 5.13^{a1}	58.33 ± 10.41^{a1}	48.00 ± 2.00^{a1}	62.00 ± 11.00^{c1}	55.66 ± 2.08^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L

3.20.19. Changes in the alanine aminotransfarase (I/U) levels of *Clarias gariepinus* during prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 89 shows the changes in the ALT levels of Ich-infested *C. gariepinus* during prolonged bath treatments. It was observed that before treatment, the ALT levels among the Ich-infested groups were significantly different ($p < 0.05$) compared to the negative control along the column whereas, at the end of the prolonged bath treatments, the ALT levels differed significantly ($p < 0.05$) especially when the treated and untreated groups were compared.

Meanwhile, the ALT levels in the healthy control were significantly reduced when compared with the negative control and the treated groups. Although at the end of the prolonged bath treatments, when the extracts groups were compared, their ALT levels increased in a concentration-dependent manner. There were significant ($p < 0.05$) increases in ALT enzyme activities among the treated groups (extracts and standard drugs) at the end of the prolonged bath treatments when compared to the pretreatment mean values in the experimental groups. But the least level of ALT was recorded in the negative control in day 7 compared to other treated groups, although in day 7, the ALT levels in the negative control was slightly higher than the standard drug treated group and extracts treated groups (D ï F), except group G that had the highest ALT levels when compared to the normal control, extracts treated groups and negative control.

Table 89: Changes in alanine aminotransfarase levels (I/U) in *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before treatment	Day 7	Day 15
A	32.00 ± 3.00^{a1}	56.67 ± 7.63^{ab1}	46.76 ± 2.88^{a1}
B	44.00 ± 7.21^{ab1}	53.66 ± 16.28^{a1}	81.66 ± 15.27^{c2}
C	38.00 ± 5.19^{ab1}	60.00 ± 5.00^{ab2}	66.66 ± 2.88^{b2}
D	42.00 ± 7.81^{abc1}	63.33 ± 2.88^{ab1}	73.33 ± 5.77^{bc1}
E	51.33 ± 4.51^{c1}	71.66 ± 10.40^{b1}	73.33 ± 7.63^{bc1}
F	49.33 ± 5.68^{bc1}	71.66 ± 2.88^{b1}	77.66 ± 4.04^{bc1}
G	47.66 ± 7.51^{bc1}	61.67 ± 7.63^{ab1}	83.33 ± 2.88^{c2}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L)

3.20.20. Changes in the lactate dehydrogenase (LDH) levels (U/L) in *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 90 shows the changes in the lactate dehydrogenase of Ich-infested *C. gariepinus* during the dip treatments. It was observed that before treatment, the LDH levels among the Ich-infested groups were not statistically significant ($p > 0.05$) when compared with the healthy control. Meanwhile, at the end of the dip treatments, the LDH levels among the treated groups, and the negative control no significant difference ($p > 0.05$) when compared to the healthy control. The LDH levels in the groups D, E and F treated with varied concentrations of *M. oleifera* aqaeous extracts, had elevated LDH levels at the end of the 1h treatments when compared to their baselines, whereas, the group G treated with the highest dose (4,500mg/l) had a reduced LDH levels together with the group C treated with the standard drug. In the negative control, the LDH levels increased at the end of the dip treatments compared to the normal control and the treated groups.

Table 90: Changes in Lactate dehydrogenase (U/L) in *Clarias gariepinus* after dip treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure).

Experimental group	Before treatment	After treatment
A	456.42 ± 57.31^{b1}	348.01 ± 97.39^{a1}
B	360.72 ± 83.66^{a1}	394.00 ± 54.74^{a1}
C	357.21 ± 51.06^{a1}	352.08 ± 59.29^{a1}
D	385.25 ± 30.24^{ab1}	412.53 ± 11.01^{a1}
E	387.84 ± 11.02^{ab1}	432.91 ± 15.25^{a1}
F	403.26 ± 23.53^{ab1}	419.90 ± 11.17^{a1}
G	425.94 ± 42.99^{ab1}	423.37 ± 3.74^{a1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (1500mg/L), E (2500mg/L), F (3500mg/L) and G (4500mg/L)

3.20.21. Changes in the lactate dehydrogenase (LDH) (U/L) in *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 91 shows the changes in the LDH levels of Ich infested *C. gariepinus* during short term treatments. It was observed that before treatment, the LDH levels among the ich-infested group did not differ significantly ($p > 0.05$) when compared to the healthy control. Meanwhile, throughout the 24 h, 48 h and 72h treatments, the LDH levels among the groups in each duration of treatments did not differ significantly ($p > 0.05$) although the negative control had a peak LDH level in the 48h treatment compared to other groups. The healthy control had reduced LDH activity in 48 h- 96 h when compared to other groups. In each treated groups, the LDH levels at the end of the short term treatments were significantly reduced especially in the group C treated with standard drug when compared to the healthy control.

Table 91: Changes in lactate dehydrogenase (U/L) in *Clarias gariepinus* after short term treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	24 h	48 h	72 h	96 h
A	341.16 ± 75.74^{ab1}	348.89 ± 138.99^{a1}	308.82 ± 63.84^{a1}	193.59 ± 89.93^{b2}	150.24 ± 50.33^{b2}
B	397.74 ± 22.33^{b1}	298.72 ± 157.52^{a2}	479.88 ± 59.84^{b1}	305.61 ± 133.30^{a1}	342.15 ± 116.05^{a1}
C	425.61 ± 28.37^{b1}	351.97 ± 88.56^{a1}	338.02 ± 37.46^{a1}	334.76 ± 10.11^{a1}	206.96 ± 116.24^{a2}
D	404.71 ± 13.68^{b1}	370.74 ± 94.96^{a1}	$368\ 74 \pm 39.92^{a1}$	328.22 ± 85.81^{a1}	275.21 ± 1080.45^{a2}
E	398.24 ± 23.09^{b1}	373.58 ± 48.25^{a1}	376.22 ± 42.51^{ab1}	343.05 ± 73.89^{a1}	271.06 ± 92.75^{a2}
F	297.09 ± 86.72^{b1}	300.31 ± 84.79^{a1}	361.78 ± 43.03^{a1}	286.88 ± 127.43^{a1}	285.06 ± 80.58^{a1}
G	371.13 ± 28.34^{ab1}	366.14 ± 50.36^{a1}	405.84 ± 89.93^{a}	310.40 ± 101.62^{a1}	268.73 ± 78.65^{a3}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$).A (control), B (infested not treated), C (infested treated with standard drug), D (150mg/L), E (250mg/L), F (350mg/L) and G (450mg/L).

3.20.22. Changes in the lactate dehydrogenase (U/L) activity in *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Table 92 shows the changes in the LDH levels of Ich infested *C. gariepinus* during prolonged bath treatments. It was observed that before treatment, the LDH levels did not differ significantly ($p > 0.05$) among the parasitized groups and the healthy control during the prolonged bath treatments. The LDH levels among the groups in day 5 did not differ significantly ($p > 0.05$) but differed significantly ($p < 0.05$) at the end of the prolonged treatments. The negative control had the highest LDH levels when compared to the treated groups and the healthy control.

Table 92: Changes in lactate dehyrogenease (U/L) levels of *Clarias gariepinus* after prolonged bath treatments with aqueous extracts of *Moringa oleifera* leaves and standard drug (fish cure)

Experimental group	Before Treatment	Day 7	Day 15
A	379.09 ± 99.27^{a1}	127.89 ± 37.75^{a2}	312.96 ± 25.59^{b1}
B	347.27 ± 47.03^{a1}	243.83 ± 65.46^{a2}	413.88 ± 84.69^{c2}
C	304.17 ± 10.82^{a1}	213.20 ± 30.40^{a2}	224.82 ± 18.87^{a2}
D	338.60 ± 55.89^{a1}	195.94 ± 86.96^{a2}	237.11 ± 29.73^{ab2}
E	336.22 ± 97.37^{a1}	248.44 ± 56.44^{a2}	210.58 ± 17.85^{a2}
F	312.64 ± 85.10^{a1}	245.75 ± 48.90^{a2}	216.95 ± 26.01^{a2}
G	299.35 ± 113.66^{a1}	243.90 ± 128.99^{a1}	239.13 ± 45.46^{ab1}

Mean values with the same alphabets as superscript in a column are not significantly different ($P>0.05$). Mean values with different numbers as superscript in a row are significantly different ($P<0.05$). A (control), B (infested not treated), C (infested treated with standard drug), D (15mg/L), E (25mg/L), F (35mg/L) and G (45mg/L

CHAPTER FOUR

DISCUSSION

4.1. Parasitological Examination

There has been a concerted efforts (Biswas *et al*, 2002; Ekanem *et al*, 2004; Chanagin *et al*, 2005; Yao *et al*,2010; Qizong *et al*, 2013) to identify suitable replacement agents or strategies for the effective control and management of *I. multifilis* infection. The continuous application of high, moderate and low concentrations of aqueous extracts of *M. oleifera* leaves and standard drug (fish cure) in this present work resulted in significant reduction in the number of *I. mulifilis* able to survive and reinfect. *M. oleifera* is a plant that has beneficial effects for humans and animals, exhibiting antimicrobial and antioxidant potentials. Ich infestation causes direct damage to fish skin and gills and leads to fish mortality. From the forgoing, it was observed that fish exposed to over 40,000 theronts in challenge trials had visible white spots within a short period of time. The newly established trophonts caused less damage to fish tissues which supports the findings of Xu *et al* (2009). The variations discovered in the trophonts present in the body smear and gill of the fish especially in some treated groups were as a result of the fact that the surface of fish is covered by a mucous layer which is a part of the defense mechanism against parasites. The mucous layer also entraps the adult parasites and making it uneasy for their removal from the body of the fish by the extracts. In addition, since infestation is the result of random encounter between the fish and the protozoal parasites, movements of water over the gills could increase the possibility of parasite contact. There is some mucous covering the secondary lamella of the gills which when stressed may not be able to entrap the parasites, which, could leave the gill even when treatment had been administered. Erkin *et al* (2012) opined that the gills, appear to be more susceptible to infestation than the body surface. This findings coincides with the

report from the present study where higher percentage cure in the body smear of the infested fish was recorded when compared to the gill. The number of trophonts that were removed from the body of fish was also as a result of the fact that the trophonts evacuated from the host tissue and settled within 2-6 hours on a substrate in the water where they formed cyst encapsulated tomonts (dividing stage). Moreso, parasites evicted from the skin and gill tissue due to the efficacy of the treatments failed to develop into tomonts and eventually died. This agreed with the reports of Hui *et al* (2012) who reported that parasites that were knocked off from the body of the host before the scheduled time for spontaneous departure, failed to develop into the tomont stage. The decreased number of trophonts recorded during the dip (1h), short term (24 -9 6h) and prolonged bath (5 - 15 days) treatments were due to varied concentrations of the extracts which reduced the *I. multifilis* adult parasites. This finding agreed with the report of (Ekanem *et al*, 2004) who reported that a Gold fish (C*arassius auratus)* infested with Ich and treated with 200 mg/L of *Mucuna pruriens* after 72h and 96h reduced the adult parasite by 90% after in vivo test. At the same time, the researchers opined that 200 mg/L of *Carica papaya* led to 100% mortality of *I. mulitfiilis* after 6h in a Gold fish.. From the present study, the efficacy of the graded concentrations of *M. oleifera* depended on the duration of treatments which led to massive reduction in the eradication of the adult parasites.

There is still paucity of literature on the efficacy of *M. oleifera* in the treatment of Ich infested fish. But further reports on the efficacy of herbal extractss in controlling/or eradicating *I. multifilis* had been reported by (Yao *et al.*, 2000). Their study showed that in vitro antiparasitic efficacy tests exhibited by sanguinarine was 100% effective against *I. multifilis* at a concentration of 0.7mg/L with LC_{50} and LC_{90} values of 0.437 and 0.833mg/L after 4h of exposure. The effectiveness of *M. oleifera* in the reduction of *I. multifilis* number on the gills in

the treatment groups was higher in comparison to untreated group for 1h, 0-96h and 0-15days. Yao *et al* (2010) suggested that *in vitro* antiparasitic efficacy test showed that the number of *I. mutifilis* on the gills in the treatment group (0.9mg/L sanguinarine) was reduced by 96.8% in comparison to untreated group at 25^0C for 48h. Therefore, *M. oleifera* may be applied as alternative to chemotherapeutants and environmental friendly drugs for controlling ichthyophthiriasis in fishes. In addition, it should be noted that effects of exposure time and concentrations may affect efficacy and effect on fish which calls for further preliminary test before a given species of fish are treated (Rzgar *et al.*, 2013).

4.2. Antiprotozoal Activity of *M. oleifera* on the Key Life Stages of the Parasite (Theronts)

Antiprotozoal activity screening of *M. oleifera* proved to be effective in controlling the infective stage of the parasite (theronts). The screening showed that aqueous extracts of *M. oleifera* had the highest activity against *I. multifilis* theronts within 24h of exposure. Ling *et al.* (2013) study showed high efficacy 1.25mg/L methanol extracts of *Psoralea corylifolia* potential in eradicating 100% infective theronts within 4h of exposure. However, Camacho (2010) reported short exposure (30min) to high doses of bronopol (i.e. 20, 50, 100mg/L) appeared to have marked effect on the survival of the tormont, cyst, and theront stages of *I. multifilis.*

In addition, prolonged, low doses of bronopol (i.e. 1mg/L) had notable effects on the survival and subsequent development of tomonts and cysts and upon the release of theronts. Similarly, there was a marked difference in the percentage of theronts surviving *M. oleifera* exposure. Although a large number of theronts were collected for this trial (i.e. 900 theronts per group) and randomly assigned to one of the five experimental containers, it could not be ascertained whether there was skilled distribution in the size of tomonts subsequently releasing

theronts (a single tormot can release between 50-300 theronts) in each population and also in the number of theronts released from each cyst. Caution should therefore be used when comparing the number of the theronts in different treatment groups as the number of parasites released from each cyst could vary widely from 250 to 3,000 tomites (Camacho e*t al.*, 2010).

The current study has shown that low concentrations of *M. oleifera* over a short period of time could have subtle effect on the mortality and survival of theronts of *I. multifilis.* Yi *et al.*(2012) reported that methanol extractss of *Magnolia officinalis* and *Sophora allopecuroides* displayed the highest antiprotozoal activity against theronts within 4h and LC_{50} values were estimated to be 2.45 and 3.43 mg/L. No report has been found on the antiprotozoal efficacy of *Moringa oleifera* plant parts on the infective stage of the parasite.

4.3. Viability of Infective Theronts after Pre-Treatment with Aqueous Extracts of *M. oleifera* for 1h.

Theronts are the infective stage in the life cycle of *I. multifilis.* Meanwhile, tomites that are liberated into the water column quickly differentiate into free swimming infective theronts. The process of differentiation from a tomite to a theront remains unclear but is characterized by the acquisition of a pyriform to fusiform shape and the development of buccal apparatus and an apical perforatorium (Camacho, 2010). The theronts must find a host during its relatively short life-span to survive and complete its life cycle. The life span of this infective stage was shown to last a maximum of 92h under low water temperature and to be inversely proportional to the ambient water temperature (Camacho, 2010).

In addition, the infectivity of the theronts in the present work showed that the Invasion of theronts into the host was as a result of the manner by which the theronts invaded the host integument which is facilitated by the excretion of sticky substances from subpellicular crystalline organelle called mucocysts. Hui *et al.* (2012) reported that active penetrations of the theronts in the host could lead to focal necrosis of the epithelial cells. It was suggested that during penetration of the theronts, they produce hyaluronidase and other enzymes. The present study supported the findings of Ling *et al.* (2013) who opined that 2.50mg/L of *P. corylifolia* reduced the infectivity of the theronts. Reports of Chu *et al*.(2010), Ling *et al.* (2012) and Yi *et al.* (2012), indicated that theronts were more sensitive to plant-active parasiticides or chemicals (Buchman *et al*., 2003), Rowland *et al.*, 2009) than other stages of Ich. Quizhong *et al.* (2013) reported that low concentrations of penatagalloyglucose at 1 or 0.5mg/L could not prevent infestation of theronts (100% prevalence), but significantly reduced infective intensity. Treatment with *M.* o*leifera* reduced theronts infectivity potentials through decrease in ich infectivity prevalence and intensity observed in the study. Generally, the fish density in a pond is much lower than that in the experimental tank, however, it took much longer time for theronts to find a fish host to invade in a pond environment in comparison with that in an experimental condition. When the search duration for a potential host is equal to or more than 1h, the theronts would almost loose the infectivity ability by the treatment of *M. oleifera* at a concentration of 0.5g/L based on the infective percentage and intensity of theronts pretreated for 1h recorded in the study.

4.4. Gross Pathology Examination

Occasionally, however, *I multifilis* only infest the gills and there are no obvious gross lesions on the body surface. Ulcers develop in the skin of some fishes which often create room for secondary infection. In the present study, some fins of the experimental fish appeared frayed because of loss of tissue between their fin rays. This observation agreed with the findings of Elsayed *et al.* (2006) who reported white spots nodules on the body of the fish and frayed fins. However, the infested fish exhibited lethargy and increased mucus production which agreed also with the report of Smith and Roberts (2010). Erkin *et al.* (2012) reported red and gray spots on the skin, fin and gill of *Cichlasoma nigrofasciatum* infested with *I. multifilis.* The change in colour of the gill reported in this present study indicated possible feeding behaviour of the parasite in the gill which affected the morphology of the gill and decreased the respiratory activities. In addition, the gills were more susceptible to infection than the skin due to little less accumulated mucus in the gill compared to the skin. Copious mucus production is defensive in function and is a physiologic response to infection. The presence of white spots around the eyes of the infested fish encountered in the study supported the findings of Woo (1995) who suggested that *C. irritans* infections could frequently involve the eyes which could lead to corneal clouding and blindness similar to those seen in ichthyophthiriasis. Earlier studies by Xu *et al.* (2009) suggested that damaged skin, lesions and ulcers were putative routes for secondary infection and subsequent manifestation of disease.

4.5. Histopathology

The nature and severity of histopathological changes seen in *I. multifilis.* Infections among *C. gariepinus* varied greatly. However, the parasite load is the major factor that

contributed to the diverse tissue changes observed in the study. These observations agreed with the report of Ventara and Paperna (1975) who opined that mild or major infestation elicit either minor or major cellular reaction. This suggested that *I. multifiliis* in minor host reactions and at the same time extensive histopathologic changes in *I. multifilis* infestations are only seen in severe epizootics or in experimental infections with large number of parasites.

The skin is made up of two major layers: epidermis, dermis and hypodermis. The dermis is separated from the epidermis by a basal membrane.The skin is one of the extremely important site of infection in fish especially icthyophthiriasis. In very mild or heavy *I. mutifilis* infestation, the only detectable pathologic changes are the presence of a few or many white spots on the surface of the fishes (Burshra, 2010). From the present study, protozoan infection in the skin of fish caused severe tissue damages. Both the epidermis and dermis were affected. The occurrences of atrophy, hyaline degeneration of muscle fibers were as a result of protozoan infection. Langdon (2006) reported that hyaline degeneration of muscle fibres may be due to proteolytic enzyme which ruptures connective tissues and loss of myofibres. Similar changes in the histology of the skin observed in this work were in consonance with the findngs by Elasayeed *et al.* (2006), Adeyemo and Agbede. (2008), Roubal *et al*. (2009). Mass infection of skin with Ich brought about anemic and necrotic condition of skin especially the epidermis, and the mucus of skin became site of collection of parasitic infection leading to liquefaction of fish muscle. However, dermal and epidermal polymorphonuclear leukocytes which infilterated on the skin were indicative of inflammatory responses. Mucus producing cells and hyperplasia observed in this study could be reactions to the increased need for muscles production in an attempt to coat body surfaces so as to prevent and/or limit contact and absorption of the extracts. The increased epithelial infiltration of neutrophils, eosinophils and lymphocytes were as a result

of congestion in the dermal lymphatics which possibly at the same time led to oedematous changes (Abalaka *et al.*, 2010). Ventura and Perpena (1985) suggested that the inflammatory reactions that occurred in the skin were due to high penetration by numerous theronts which lead to increased epithelial cell hyperplasia due to generalized increase in the number of mucus cells in the skin. The cellular proliferation could be a defense mechanism. The extensive cellular reactions observed in the skin of fish infested with *I. multifilis* were as a result of hypersensitive reactions. The skin (pigmented and non-pigmented parts) reaction by parasites exposures is normal and naturally defensive. This was in agreement with the report of (Abalaka *et al*., 2010) who opined that the skin reactions to parasite exposures were naturally spontaneous and defensive. The destruction of the epithelial cells observed in the present study suggested the impact of the infective stage of parasites (theronts) in and out of the skin which caused damage on the host. The dermatitis observed in this study was in agreement with the findings of Forough *et al.* (2012) who reported dermatitis and purulent bronchitis which were observed in Oscar and Discus infested with Ich. Haemorrhage in dermal layer of the skin recorded in this study was as a result of observed massive aggregation of red blood cells in the skin tissue due to parasite invasion. Woo (1995) reported evidence of haemorrhage occurring in the skin of fish due to external parasite infection. Moreover, abscess formation, bundles of fibres fused together leaving small spaces along with hydropic and vacuolation reported in this present work corresponded to the findings of Erkin *et al*. (2012) who reported that mature parasites entering between epithelial layer of the skin increased to the basal membrane which appears necrotic, hydropic and vacuolated. Toksen (2000) reported that Rainbow trout experimentally infested with *I. multifilis* showed increased in number of cells and mucus were found in the skin of the fish. The presence of some immature parasites (trophonts) that were proximately lodged in the epidermal layers

were adjacent to the basement membrane of the epithelial layer, whereas, the surrounding tissues showed no evidence of damage. This was due to the fact that trophonts develop within the integumentary epithelium, always above the basal membrane and at the same time created a tissue space in the epithelial layers.

The distortion of muscle tissue in the present study may be due to proteolytic enzymes secreted by protozoans. This finding agreed with Robertson *et al*. (2006) who reported dessolution of muscle of salmonids infested with *Ichthyobodo necator* and at the same time that certain protozoans could produce proteolytic enzymes which brought about the structural changes in the muscle tissue. A layer part of the skin appeared deformed with several round spaces, which indicated dissolution of muscle tissues due to penetration by the parasites to the superficial and deeper layers of the skin. Abnormalities in the layers of dermis and epidermis were obvious while some degeneration of muscle fibres were due to severity of infestations in the skin caused by *I. multifilis.* Ewing *et al*. (1988), however, reported that the number of trophonts within a particular section of epithelium usually increased during infestation and postulated multiplication of parasites within vessicles. The presence of smaller parasites observed in the present study indicated that smaller trophonts never reached maturity while some reached maturity. This supported the finding of Elsayed *et al*. (2006) who reported small and immature trophonts in siamese sharks infested with Ichthyophthiriasis. Also, the non formation of somatic cysts by the fish body around the parasites that were typically encountered in typical Icthyophthiriasis, was due to non-specificity of the parasites strain rather than host resistance. The separation of muscle fibres, distortion of muscles observed in the present study may be due to mass infection of skin with Ich which made the mucus of the skin to become collection of parasitic infection which led to liquefaction of fish muscle. The parasites at the same time caused

copious effusion of mucus which disrupted respiratory functions of skin and ionic exchange of blood in fishes.

An extremely important site of infestation was the gill because the trophonts within the gill epithelium were a major factor in the lethal effect of infestation. Fish gills participate in many important functions such as respiration, osmoregulation, and excretion. They remain in close contact with external environment and are particularly sensitive to changes in the quality of water (Mousari *et al.*, 2012). In the present study, the theronts were attached to the gills at the middle or base of the gill lamellae. The increased number of the parasites in the gill was potentially harmful because of the various histopathological alterations observed in the present study which possibly caused impaired respiration. This was reported to kill or damage fishes, and may slightly reduce their productivity (Mousari *et al.*, 2012). Hyperemia observed in the gills in fish in the present study was in agreement with the findings of Smiths and Roberts (2010). Adult parasite trophonts observed at the base of the primary filaments as well as the gill epithelia may be due to trophonts penetration and displacement of the interlamellar epithelium until they finally found their way to the basement membrane. Similar results were also reported by Woo (1995) who opined that mature trophonts may occupy three or four lamellae in the gill and as such caused gill malfunction in fish. The trophonts observed in the gill of the infested fish appeared with largeï C shaped macro nucleus. In the gill, the growing parasites caused distortion of the lamellae, severely fused lamellae, epithelial hypertrophy, atrophy, and lots of inflammatory cells which may be due to destruction or hypersensitivity reaction. The exit of mature trophonts from the gill epithelium left significant holes in the tissues which contributed to osmoregulatory problems.

In this study, lamellae fusion mainly involved two or three neighboring lamellae indicating that it was likely to be parasite-induced. This agreed with the report of Nyasha and Maxwell (2006) who reported that fusion of adjacent lamella was normally related to histopathological lesions such as hyperplasia if it is systemic (i.e. occurs in vast areas) or parasitic if two or more neighbouring lamellae fused together. Epithelial lifting of the secondary lamellae observed in the study was due to infiltration of fluid made oedematous space between epithelium and basement membrane. Similar to hyperplasia, this kind of lesions inhibited the respiratory gas exchange by increasing diffusion distance and decreasing interlamellar distance. Moreso, fusion of the adjacent secondary lamellar was also as a result of hyperplasia of epithelial lifting and mucous cells proliferation or in some cases, hypertrophy of epithelial cells or due to some anomalies like proliferation of epithelial cells which led to lamellae fusion. The appearance of extended lamellar oedematous spaces, sometimes containing infiltrated cells, seemd to implicate a protective and osmoregulatory manifestation of the gills. Also, the occurrence of aneurysm observed in this study may be used as a sensitive and reliable biomarker of parasite invasion in the gill. These histopathological changes were reversible especially among the groups treated with standard, but the only exception was aneurysm which is normally classified as a severe condition as reported by Woo (1995). The curling/clubbing of the lamellae reported in this work may be due to progressive infection which led to epithelial cell proliferation and filling up of the inter-lamellar space until the lamellae were completely cornified or clubbed. This also agreed with the similar findings by Woo (1995).

The histopathological changes observed in the gill structure which occurred after exposure of the fish to the parasites and subjected to immersion bath treatments may have

seriously reduced the oxygen diffusing capacity of the gill so that death possibly resulted primarily from insufficient oxygen uptake.

4.6. Proximate Composition

Fish is known to be one of the cheapest sources of crude protein and other essential nutrients required in human diets. The study and importance of the nutritional compositions and their mineral elements is very vital because it plays an important role in some metabolic processes which are known to be indispensible to all living things. However, considering the various health risks and the nutritional benefits associated with Ichthyophthiriasis disease, it was imperative to assess the fish proximate composition so as to establish the safety level of the table sized species prior to human consumption. No account is available on the effect of herbal extractss on the proximate compositions of Ich-infested fish. From the foregoing, it was observed that several variations occured in the proximate composition of Ich infested fish especially in the protein, fats and carbohydrate contents. It cannot be categorically stated that the observed differences in their proximate composition were influenced by the parasitic infestation. This is because the level of parasitic infestation observed was moderately high and only some of the infested fish had lesions and frayed fins in their body which exposed them to secondary infections. At the same time, because there were no deep lesions observed on the body of the infested fish, the variations in their proximate composition may not be due to infection. In contrast, it cannot be overruled that heavy infection can affect the nutritional composition of the fish. This is because the parasite could invade the body host and then penetrate within 5 minutes to the basal layer. At the same time, the trophonts created a tissue space in the epithelial layers. The parasites within the vesicles appears as white spots and occasionally several parasites

occured within the same vessels, whereas, the number of trophonts within a particular section of epithelium increased during infection. The more they increased in number the more they created tissue spaces and the more they caused severe lesions. If not noticed, they eat deep into the tissue and predispose them to secondary and opportunistic pathogens and at the end may affect the nutrient composition of the fish. The need to have a knowledge of the proximate composition of fish in order to make best use of them as food and how to control the ich infestation or develop a better way of controlling the parasite is paramount. There is serious paucity of literature on the proximate composition of Ich-infested fish.

4.7. Haematology

Blood parameters can be useful for the measurement of physiological disturbances in parasitized fish and thus provide information about the level of damage in the host and the prognosis of the disease. Research into the clinical haematology of fish may enhance the knowledge of pathology which is needed to improve diagnostic practice and to select the best preventive procedure.

Movahed *et al* (2012) reported that parasites can act as stress factors which affect haematological parameters such as reduction in RBCs count, Hb value and packed cell volume that may often cause anaemia. Present study examined the basic haematological alterations for monitoring health conditions of Ich-infested fish. There is still paucity of literature in efficacy of *M. oleifera* leaves extracts in the haematological parameters of Ich infested fish.

Clarias gariepinus were parasitized by *I. multifilis*, no changes were observed in the white blood cells after prolonged bath treatments but there were significant changes ($p < 0.05$) in the WBCs levels during the dip and short term treatments. The elevated WBCs count recorded

throughout the bath treatments was as a result of defense mechanism against the parasite infestation of *I. multifilis*. Absence of alterations in WBCs levels during the prolonged bath treatments might be indication that leukocytes count in the course of the disease were dynamic and probably related to it severity and these explained why Ich rendered fish very susceptible to other pathogens. This finding agreed with report of (Witeska *et al.,* 2010) who opined that *Oreochromis nilolticus* infested with Ich had a decreased WBCs count and phagocyte activities. The observed drop in the leukocyte count in fish with severe symptoms of Ich in the present study differed from the result obtained by (Hines and Spira, 2004).

However, the observed increase in the infested not group may be due to possible sequestration by the WBCs in combating the invasion of the parasites, Luciana, *et al.* (2006) reported that ectoparasites infestations represent an important challenge to fish which led to an activation of the cellular defense mechanism and fish with moderate symptoms of disease usually exhibit increased WBCs without alterations in differential count.

The observed decrease in the erythrocyte levels of the infested not treated group indicated slightly anemic conditions throughout the varied bath treatment exposures although, a markedly increased RBCs level were recorded among other treated groups during the dip, short term and prolonged bath treatments.

The reduction in RBCs levels of the infested fish observed from this study supported the findings of Tavares ï Das *et al.*(2002) and Movaheed *et al.* (2012). In contrast, Kurovskaya and Osadchaya (1993) did not report anaemia in comon carp infested with *I. multifilis*. Similarly, Witeska *et al.* (2010) reported anaemia and a shift towards phagocytes in *O. niloticus* with gill

ichthyophthiriasis and saprolegniasis. Abdel *et al.* (2014), reported anaemia in rainbow trout *(Oncorrhynchus mykiss*) and Chub (*Leuciscus cephalus*) infested with *I. multifilis*.

The decrease in the number of circulatory red blood cells probably reflected the physiological functioning of haemtopoietic system, which was considered to be the most sensitive indicator. The alterations in the erythrocyte count produced erythropenia which could be attributed to an increased fragility which led to the shortening of the life span of the erythrocytes. However, it was presumed that the decrease of total erythrocyte count may be attributed to the parasite invasion especially in the gill tissues were numerous RBCs were recorded keeping for possible haemorrhage that lead to an anemic condition. The Ich parasite presents in the body influenced the malfunctioning of the haemopoeitic system, and the haemopoeitic tissues failed to release the blood cells which were subsequently released into the blood stream. Increased PCV and Hb could be due to Ich sensibilities to *M. oleifera.*

Moreover, the reduced packed cell volume and haemoglobin in the infested untreated group agreed with the findings of Tavares ï Dias *et al*. (2008) who reported decline in the PCV and HB in *Oreochromis niloticus* infested with Ich. Meanwhile, the blood parameters including, PCV and Hb were higher in healthy *C. gariepinus* while other treated groups exposed to varied bath treatments, had significantly higher PCV and Hb at the end of the various bath treatment exposures. This observation supported the findings of Witeska *et al*. (2010). Decreased PCV was also observed in channel cat fish exposed to *Edwardisiella ictalurus* (Xu *et al.*, 2012). The use of differential leucocyte count is a reliable haematological index. The different types of leukocytes had been studied under the categories of granulocytes characterized by the presence of specific granules in the cytoptasm and stained very well with different stains such as lugol iodine and

0.01% neutral red stain, and granulocytes lacking the specific granules and lymphocyte percentage has been significantly increased causing lymphocytosis in fish infested with Ich (Abdel *et al.*, 2014).

Differential leukocytes count play roles in assessing the health status of fish. Lymphocytes are the primary components of the bodyôs immune system. They are the source of immunoglobulin and of cellular immune response. As a result, they play an important role in immunologic reactions. From the present study, increased significant lymphocytes were recorded among the treated groups, the infested untreated and the normal control at the end of the varied bath treatment exposures. Possible lymphocytosis was observed among the treated groups. Abdel *et al*. (2013) reported increased lymphocytes and thrombocytes in rainbow trout and Chub infected with Ich parasite.

Neutrophils are types of white blood cells that the body uses to fight infection. High neutrophils levels are often caused by infections, whereas, some drugs and health conditions could cause them as well. In many instances, increased number of neutrophils is a necessary reaction by the body, as it tries to heal or ward off an invading foreign substance (Abdel *et al*., 2013).

In the present study, during the dip treatments increased neutrophils levels were observed at the end of the Ih treatments though the increase was not statistically significant ($P > 0.05$). The possible increase observed in the neutrophils may be due to their phagocytic activities. Infections by bacteria, virus, fungi and parasites may increase the number of neutrophils in the blood (Luciana *et al.,* 2006). Increase in neutrophils number in Tilapia was inferred to be parasitism which affected neutrophils population in the circulatory blood by inducing more production

which enhanced the host responses (Hines and Spira, 2004). The decrease in the neutrophils count observed during the short term and prolonged bath treatments among the treated groups was an indication of possible neutropenia while increased neutrophil is called neutrophilia due to polycythemia. The decrease in neutrophils was due to inhibition in the functionality of their phagocytic activity by the *I. multifilis* parasite load in the body and gill of the fish. Hines and Spira (2004) reported the presence of myelocytes and metamyelocytes including and a single blast cells in the common carp infested with icthyophthiriasis which was probably due to phagocytes quickly migrating to the infested tissues where they reached functional maturation, and their elevated concentrations in the blood indicated a high rate of their release from haematopoietic tissue. Detected haematological alterations indicated partially compromised health status that may led to reduced fish resistance to other challenges. Some hematological variables might vary according to fish handling, water conditions of source of feeding etc. Moreover, these alterations were directly related to the degree of infestation and number of pathogens on/in the host. Ectoparasite of fishes may induce haematological alterations (Luciana *et al*., 2006) in the fish due to ion regulatory or respiratory disturbances that imply increase in energy consumption to restore homeostasis instead of other physiological functions.

4.8. Blood Electrolyte

Electrolyte concentrations are regulated by interacting processes, such as absorption of electrolytes from surrounding medium through active mechanisms predominatly at the gill, control of water permeability and selective reabsorption of electrolytes from urine. Any alterations in one or more of these processes results in a change in the plasma electrolyte composition. These ions play a vital role in several body functions. Monovalent ions such as sodium, potassium and chloride are involved in neuromuscular excitability, acid base balance

and osmotic pressure. Physiological changes of the blood electrolytes (Na^+, Cl^-, k^+ and HCO_3^-) are typical changes found in catfish especially channel catfish and they are very resistant to loss of electrolytes when exposed to stressors.

From the present study, the sodium levels among the groups treated with varied concentrations of *M. oleifera* after, dip, short term and prolonged bath treatments showed that the varied concentrations of the extracts and the standard drug elicited a significantly higher sodium levels. In the body of the fish, the elevated levels of sodium were higher in the treated groups compared to the baseline normal control leading to possible hypernutremia in the fish.

There is serious paucity of literature in the blood electrolytes levels of Ich infested *C. gariepinus* treated with *M. oleifera* aqueous extracts. However, the presence of excess sodium in relation to water from the present investigation was due to the fact that sodium is usually found outside cells where it helps regulate the amount of H_20 in the body. So, the regulation of water in the body was highly increased leading to increased sodium level and decreased water levels which possibly caused cells malfunction or dysfunctions.

The decreased sodium levels observed in the present study among some treated groups may not possibly elicit hyponatremia condition may impair the functions of sodium in regulating water in the body. This finding supported the reports of Davis *et al*. (2002) who opined that plasma electrolytes decrease in many fish species due to stress. It could be deduced that the Ich infestation in the *C. gariepinus* caused a shift in the sodium balance of the fish. Abdel *et al*. (2014) reported decreased serum sodium concentration in rainbow trout and club fish infested with Ich. Tavares *et al*. (2007) reported changes in ion balance of fish parasitized with crustacea parasites and other parasites. However, the increase in sodium levels in the present study,

conicided with the findings of Tavares *et al*. (2007) who reported elevated sodium levels in Hybrid tambaeu parasitize by *Dolops carvalhoi.*

Potassium is found mainly inside the body cells. Small amounts are also found in blood plasma and plays role in regulating the heart rhythm and ability to counteract sodium. From the foregoing, it was observed that potassium levels increased significantly ($P < 0.05$) at the end of the dip treatments 48h ï 72h, short term treatments and day 5 ï day 15 prolonged bath treatments among the groups. The increase may be due to hyperkalemia in the body of the infested fish during the treatment. This was due to the fact that potassium was is frequently excreted by the kidneys, so the disorder that hampers the functions of the kidneys could lead to elevated potassium levels. This result is in consonance with the findings of Tavares *et al*. (2007) who opined that ectoparasites infestations can lead to osmoregulatory disturbances but reported decreased potassium levels in Hybrid tanbaeu parasitized by *D. carolhoi.*

Chlorides play a role in helping the body maintain a normal balance of fluids. It is the major anions (negatively charged ion) found in the fluid outside the cells and in the blood. From the present study, elevated chloride levels were evident among the different bath treatments (dip, short term, and prolonged bath) used in eliminating Ich infestations in the experimental fish and the concentrations used. The increased chloride levels among the treated groups and the negative control were higher than the normal baseline control depicting possible moderate hyperchloremia. However, the standard drug treated group maintained normalized chloride levels close to the normal control. Meanwhile, the sodium levels in the infested fish and among the treated groups were not higher than the chloride level and may be ruled out as a non-cause of higher chlorine seen among the extracts treated groups but may be due to possible renal failure or

loss of body fluid. The time-dependent decrease observed among the treated group may be due to the fact that the chloride levels in the body of the infested fish tried to keep the acid-base balance in the body. Decline in serum osmolality and $Na^{+}$and Cl^{-} concentration in *Onchorhyncus mykiss* and *Leuciscus cephalus* infested with Ich was reported by Abdel *et al*. (2014). Davis *et al*. (2002) reported that the response to stress by fish is often characterized as being composed of a primary and secondary phases. The secondary phase is the physiological consequences of the primary responses represented by chloride which is involved in retaining osmotic pressure and acid-base balance. There is an egress of sodium and chloride as a mitigating function for maintaining osmotic pressure.

The decrease in the chloride serum observed in the present study was an indication of stress response by Ich infestation. Hence, these results supported the suggestion of Tavares ï Dias *et al*. (2007) who asserted that infestation with crustacean parasites may elicit an osmoregulatory response in the host, which needed to maintain homeostasis since the maintenance of a consistent internal environment was essential for normal cell function in plurocellular organisms.

The bicarbonate acts as a buffer to maintain the normal levels of acidity (pH) in blood and other fluids in the body. It is measured to maintain the acidity of the blood and other fluids in the blood. The declined levels of the bicarbonate at the end of the dip, short-term and prolonged bath treatments was because the level of Ich infestation in the fish especially the gills reduced drastically during the treatment periods. The acidity in the blood decreased and the decrease was time-dependent throughout the immersion treatments. There is no available literature to support these findings after ich-infestation treatments with *M. oleifera*. But the possible disruptions of

the bicarbonate levels before treatments may be due to the Ichthyophthiriasis which interfered with respiratory functions in the fish.

4.9. Antioxidants

Parasites use the host as a habitat and nutrition source and adapts to its peculiarities. Parasites affecting the metabolism of infested fish stimulate oxidative stress manifested by increasing free radicals and peroxide processes moderating the host antioxidant status. Changes in the antioxidant enzyme activities in infested fish depend on the species of host and parasite and on the life cycle stage of parasite. As a result of infection synthesis, reactive oxygen species (ROS) increases in the host organism causing parasites elimination which can lead to the inhibition of the antioxidant enzyme activities. Parasitic infection can lead to the inhibition of antioxidant enzyme activities associated with the production of ROS by macrophages at the infection site in order to eliminate the parasite.

The decreased superoxide dismutase (SOD), catalase (CAT) activities in the gill and muscle of fish with average and high intensity of infection could be the result of their inhibition with ROS of host macrophages and the high content of parasites metabolites, which caused oxidative stress and prevented greater danger for fish health. At the same time no significant decrease in SOD, CAT can be the host adaptive response directed to the organism functioning maintenance at the high intensity of infection (Skaratovskaya *et al.,* 2013).

Bello *et al.* (2000) suggested that the fresh water fish *Rhandia quela* response to infection by *Clinostinum detrinctum* could involve ROS intermediates and therefore, induced oxidative stress,

but without significant differences in the SOD and CAT activities between healthy and parasitized fish. *Abranis brana* infected with plerocercoids of *Ligual intestinalis* were characterized by high content of MDA and low antioxidant activities, which was associated with an increased stress under the influence of parasites and the development of oxidative stress.

Under normal physiological conditions, animal cells produce reactive oxygen species (ROS) such as H_20_2, $0H^-$ and O^-, which may damage cellular components leading to cell death. When the rate of ROS generations exceeds that of their removal, oxidative stress occurs. Catalase activity plays an important role in antioxidative defense of the cell by reducing H_20_2 and converting it into H_20 and 0_2 and is critical for the process of scavenging free radicals (Bello *et al*., 2000). In the present study, the catalase activity in the gills of the Ich infested *C. gariepinus* among the groups had a fairly elevated CAT activity during the dip treatments while the CAT activity in the muscles gave same elevations in some treated groups. During the dip treatments, the CAT activity in the muscles was lower in the positive control group when compared to the extracts groups. The increased level of CAT activity encountered during the dip treatments among the extracts treated groups and standard drugs may be due to triggers of antioxidant enzymes.

However, there is serious paucity of literature in the antioxidant status of fish infested with ichthyophthiriasis. Meanwhile the different concentrations and varied duration of treatments elicited various elevated CAT levels in the infested *C. gariepinus* especially in the prolonged bath treatments. Although, the CAT activities in the gill and muscle of ich infested *C. gariepinus* during short term treatment were reduced among the treated groups and the control when compared to their baselines. This depicted that the reductions in CAT activities in the gills and

muscles of the ich infested C. *gariepinus* were time-dependent, although their CAT mean values varied significantly when looking at the reduced concentration treatments and duration of treatments. Suarav *et al*. (2012) reported that catalase activity showed similar trend with little activity recorded in the negative control group with elevated CAT activity in the serum of the Argulus-Infested *Carassius auratus* treated with azadirachtin. The decreased CAT activities may be due to ROS which affected the antioxidant defense mechanism, thereby decreasing the CAT activities in the gill and muscles.

There was a dose dependant, significant depletion in the muscles of SOD activity into the experimental fish and no significant depletion in the gills of the infested fish over time. The depletion in SOD found in this study throughout the immersion treatments (dip, short term and prolonged bath) suggested that oxidative stress was induced during parasitic infestation in the experimental fish which led to the significant decrease in the activities of SOD both in the gills and muscles. SOD catalyses the destruction of superoxide radicals and itôs activities is an indication of the tissues to cope with oxidative stress (Misra, 1972). When there is high increase in oxidative stress, like in this study, the defense capabilities against ROS collapsed. Suarav *et al*. (2012) reported that the decease in the SOD and CAT may be attributed to an influx of superoxide radicals. Contrary findings was reported by (Suarav *et al*., 2012) who opined that argulus- infested fish had an elevated SOD levels which may be attributed to lower oxidative stress in the Azadirachtin treated groups. However, SOD provides the first line of defense against oxygen derived radicals. SOD activity decreases oxidative stress by dismatation of O_2^- (Suarav et al,). The observed increase in the SOD activities in the gills and muscles of ich infested *C. gariepinus* in group B (infested not treated) during prolonged bath treatments may reflect compensatory mechanism to increased oxidative stress due to the parasitic infestation.

Gluthathione peroxidase is an antioxidant enzyme that functions in the scavenging and inactivating hydrogen and lipid peroxides, thereby protecting the body against oxidative stress (Sies, 1996). It plays a very crucial role in, as it is a free radical scavenger that protects the body from oxidation. It also catalyses the reduction of hydroxyperoxides by gluthathione. It protects against the damaging effect of endogenously formed hydroperoxide. The observed decrease in the GPx activities in the *C. gariepinus* infested with *I. multifilis* and treated with different graded concentrations of aqueous extracts of *Moringa oleifera* under dip, short term and prolonged bath treatments may be due to increased free radicals. Moreso the observed elevated levels of GPx may be due to the sequestering potentials of GPx in eliminating and scavenging free radicals from the body of the Ich infested fish. No literature was available on GPx activities on *I. multifilis* infested fish treated with *Moringa oleifera* leaves or any other plant materials Suarav *et al*. (2012).

Meanwhile, the assessment of lipid peroxidation is usually performed by analyzing secondary oxidation products such as malondialdehyde (MDA). This distinctive compound has been employed as a model compound for studying secondary degradation products of lipid peroxidation. Lipid peroxidation results when fatty acids come in contact with ROS, to produce series of reactive aldehydes, including MDA. From the present work, the observed changes in the MDA levels in the ich infested fish during the dip, short term and prolonged bath treatments may be due to reduction in the level of free radicals production, whereas, the slightly increased MDA level may be due to small increase in the production of free radicals and indirectly indicated small degree of oxidative stress inducd by the parasite (Mohen *et al*., 2010). There was serious paucity/or no literature on the MDA changes in Ich infested *Clarias gariepinus.*

Myeloperoxidase (MPO) is a hemoprotein secreted during activation of neutrophils, which plays an important role in defense of the organism. MPO is stored in primary, azurophilic granules of neutrophils. It is a major component of the broad bactericidal armamentarium of neutrophils. It utilizes hydrogen peroxides during respiration burst to produce hypochlorous acid (Kumar *et al.*, 2012). From the present study, the elevated levels of the MPO activity present in the gills and muscles of Ich infested *C. gariepinus* among the treated groups when compared to the control may be due to sequesteration of the presence of stress contaminants such as chemicals and parasites. Although the decrease in the MPO levels in the muscle and gills of the negative control during the dip treatments may be due to presence of contaminants of stress especially the Ich parasites which supported the findings of Kumar *et al*. (2012). Harikrishan *et al.* (2012) reported significant decreased MPO activity in all the treated groups compared with infected ones in *Aeromonas hydrophila* infected *Cyprius carpio* treated with herbal medicine.

Subsequently, throughout the short term and prolonged bath treatments, the MPO activity decreased in the gills and muscles of negative control (group B) when compared to the normal control and the treated groups. The decreased MPO activity had earlier been stated to be due to high level of parasitic infestation that concomitantly induced stress in the fish. Moreso, in all the other treated groups, there was reduced stress due to increase secretion of MPO which usually activates neutrophils and played important role in the defense of the Ich infested *C. gariepinus*. There was still paucity of literature on MPO levels in fish infested with protozoan parasites especially *I. multifilis* and treated with *M. oleifera*. This was the first time MPO has been reported in the ich-infested *C. gariepinus* exposed to varied immersion treatments.

4.10. Biochemical

Parasitic infection could cause biochemical reorganization of metabolism in infected tissues, directed to selective uptake of nutritional substances and could lead to serious structural and functional changes in the organs (Woo, 1995).

Proteins are the most important component in the serum including plasma proteins which are termed as circulating mobile proteins. The serum proteins are divided into two groups; albumin and globulins. The liver synthesizes albumin and creates an osmotic force that maintain fluid volume within the vascular space (Harikrishnan *et al*, 2012). The albumin is an easily available protein reserve and a protein transporter. The gamma globulin fraction is the source of almost all the serum biochemical active protein of the blood. Globulins like gamma globulins, are essential for maintaining a healthy immune system. Albumin is quantitatively the most important protein in plasma synthesized by the liver and is a good indicator of hepatic function. Total serum protein measures the quantity of albumin and globulin present in the blood. The parenchyma cells of the liver are responsible for synthesis of fibrinogens, coagulation factors and albumin while the immune system synthesize globulin mainly (Wiegerties *et al.*, 1996).

Since gamma fraction makes the largest portion of globulin, it can be inferred that subsequent enhanced globulin level in the treatment groups within the treatment patterns (dip, short-term and prolonged baths) may enhance the immune response and preparatory stages for stress mitigation of fish. The decrease in serum protein of ich infested fish may be due to some degree of dysfunction under the conditions of diseases. Under the stress condition, the gills became leaky to water and ions which led to disturbances in osmoregulatory balance. So the decease in serum

protein and globulin may be due to haemodilution, and osmoregulatory imbalance (Kumar *et al*., 2012).

From the present study, the relatively high level of total protein and globulin recorded might suggest possible secondary infection to *C. gariepinus* or immunomodulatory activity of *M. oleifera*. This coincided with the result of Kumar *et al.* (2012) who reported increased total protein and globulin levels due to immunomodulatory activity of azadiracthin extracts in argulus infested fish. Similar findings regarding total protein was been reported in Olive flounder infected with *Philasterides dicentrachi* (Harikrishnan *et al.,* 2012).

The main reason for increased total protein levels among the Ich-infested group before treatment was stress. *C. gariepinus* infested with *I. multifilis* had increased total protein while Harikrishnan *et al.* (2012) reported no significant alteration in total protein in gold fish infected *Aeromonas hydrophila*. The observed reduced total protein recorded during the short term treatments could also be caused by malabsorption, impaired liver and dehydration. The reduced total protein could be due to hepatocellular damage which decreased protein production in the liver. The concentration dependent reduction of serum protein during the short term treatment reflected the concentration dependent liver protection of *M. oleifera* and the standard drug during the prolonged bath treatments. The increased total protein could be seen as a useful index of the severity of cellular dysfunction in liver diseases. The increased albumin level observed during the dip treatments could due to the fact that stimulation of protein synthesis was advanced as a contributing self healing mechanism, which accelerated liver regeneration process. *C. gariepinus* infected with trichodina had significant decrease in total serum albumin and globulin (Osman *et al.,* 2009).

Aspartate amino transfarase activity (AST) is also known as serum glutamate oxaloacetate transaminase (SGOT) and alanine amino transfarase (AIT) is also known as serum glutamate pyruate transaminase (SGPT). Both enymes are present in the cytosol of hepatocytes. However, AST is also found in the mitochondria. It is present primarily in the liver and to a lesser extent in kidney and skeletal muscle. SGPT is found in all body tissues especially heart, liver and skeletal muscle. Both enzymes are specific to liver but SGPT is found in higher concentration in the liver than in other organs. Increased activity of SGOT and SGPT in serum of the Ich infested group before treatment during the dip, short-term and prolonged baths suggested liver cell damage and leaching of the enzymes in blood (Manteiro *et al.,* 2006). Wikaler *et al.* (2007) reported higher level of SGOT and SGPT in argulus infested group and post-treatment groups with partial elimination of ectoparasites. The biological stress and higher concentration of organic compounds had negative impacts on liver tissue and this was in conformity with reports of higher SGOT and SGPT levels by Roe *et al.* (2006). In *Labeo rohita* fed with diet containing herb, *Achyranthus aspera,* Kumar *et al.* (2002) reported in post in vivo study, high levels of SGOT and SGPT values found in Argulus-infested negative control group while low levels were recorded in 15mg/L azadiracthin treatment group, revealing the stress dimination by reduced protein catabolism and hepatocellular damage. The decrease in SGOT and SGPT at the end of the treatments (dip and short term) in the treated groups when compared to the negative control may reflect the effectiveness of standard drug and *M. oleifera* in improving hepatic injury. Meanwhile, the elevated SGOT and SGPT recorded may be due to liver damage caused by leakage of the enzyme into circulation after cellular damage. But in contrast to the SGOT and SGPT levels in extracts treated groups, the increased activity of SGOT and SGPT at the end of the prolonged bath treatments compared to their baselines (before treatment) may be due to the

mechanism of action of the *M. oleifera* extracts in preventing intracellular enzyme release and itôs membrane stability and antioxidant effects. Although the groups treated with standard drug had reduced SGOT and SGPT when compared to the extracts treated groups and the negative control. Also, the increased SGOT and SGPT levels during the prolonged bath treatments may be due to the fact that when *M. oleifera* was administered in a low concentration, there may be an enzyme enhacement that led to hepatic injury in the ich-infested fish. However, the elevated SGOT and SGPT in the treated groups and the negative control were dependent on the duration and concentrations of the standard drug and extracts used.

Urea and creatinine concentrations are used for the assessment of renal sufficiency. Higher than normal level of serum urea and creatinine are indications of deficiency in renal function. Thus the increase in serum urea concentrations with concomitant increase in serum creatinine concentrations in the infected untreated fish, suggested that the normal functioning of the kidneys had been compromised. However in the present study; elevation of urea levels in the Ich-infested treated fish may be due to gill dysfunctions beacuse the urea was excreted mainly through the gills. These findings may be attributed to the inflammatory reactions and intoxication produced by *I. multifilis* parasites in the affected fish. This observation agreed with the reports of Mahmoud *et al.* (2011) who reported elevated urea levels in some freshwater fishes infested with external parasites. Meanwhile it is imperative to note that urea is excreted in fish mainly though the gills, so the elevation in the urea level in the different treatment patterns applied in the present study elicited gill dysfunction especially among the negative control. This finding was supported by the reports of Osman *et al.* (2009).

The elevation of the urea levels in the negative control suggested that *I. multifilis* parasites elicited the increase. This indicated that exposure of fish to parasitic infection wass more powerful in stimulating the activities of the urea. Similarly, Mamouud *et al*. (2011) reported elevated creatinine and urea in the infested fish species, *Oreochromis niloticus* and *Clarias gariepinus* with external parasites than the non-infected fishes in different localities. The fairly increased levels of serum creatinine in the current study may had been induced by glomerular insufficiency, increased muscle tissue catabolism or impairment of carbohydrate metabolism. It is worthy of note that during the varied treatment patterns, the creatinine levels increased mostly at the end of the treatments among the groups. Similar increase was observed also in the urea levels in the treated groups when compared to the treatment patterns (immersion treatments) used.

Lactate dehydrogenase is the terminal enzyme of the glycolysis pathway. LDH converts lactate to pyruate in the presence of coenzyme NADH that is converted to NAD^+. Thus, lactate dehydrogenase helped in maintaining the glycolysis cycle by supplying NAD^+. In the presence of enough oxygen, pyruvate enters the krebs cycle, but when there is deficiency of oxygen in the tissue, pyruvate is converted to lactate.

In the present study, the LDH activity increased during the dip treatments but during the short term treatments, among the extracts treated and standard drug, LDH activity were decreased compared to the negative control that had elevated LDH. The decreased LDH level in treated groups may be due to reduction in stress, although the increase in LDH observed in the negative control may also be due to stress caused by the *I. multifilis* parasite. This report supported the findings of Vijayara and Roa (1986) who opined that increased LDH activity may be due to

stress induced by the parasites, temperature, starvation or confinement stress. Increased LDH indicated metabolic changes i.e. the glycogen catabolism and glucose shift towards and led to the formulation of lactate in the stressed fish, primarily in the muscle tissue. Increased plasma LDH activity may therefore reflect an increased dependence on anaerobic carbohydrate metabolism of the exposed fish.

Meanwhile, the decrease in LDH after treatments with the extracts and the observed increase after treatment could be due to the different concentrations, mode of administration and the physiological status of the experimental fish. Among the treatment patterns and concentrations used, the short term and prolonged bath treatments showed the most potent effect in reducing the level of LDH after the duration of the study. The observed increased in LDH in this study could also be as a result of enzyme inhibition mainly due to increased membrane fluidity as a result of ROS involvement which led to enzyme leakage into circulation. The regulating effects of *M. oleifera* and the standard drug on LDH could in turn normalize microsomes, lysosomes mitochondria and plasma membranes permeability and integrity which led to the reduction of the liver enzymes towards itôs normal level.

CONCLUSION

Ichthyophthirius multifiliis or ówhite spotô is a wide spread ciliate parasitic protozoan that is commonly encountered in most cultured freshwater fin fish species. This present findings offered a range of treatment method options for use in commercial farms systems not only for the control of Ich infestations but also other freshwater and marine protozoan's diseases. There is need for proper licensing of environmentally friendly non-chemotherapeutants to control parasite infection in food fish farms. In addition, the most effective management strategy to use against Ich, however, may be site/farm specific and require a combination of the different treatments considered in the current study.

Finally the bath treatments demonstrated efficacy of novel non-chemical treatment *Moringa oleifera* at high and low concentrations in eliminating and reducing the survival of free living stages, especially theronts and trophonts. The dip treatment appeared to be the best in completely eliminating the adult parasites in ich infested *Clarias gariepinus* when compared to the short and prolonged bath treatments.

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