STUDIES ON THE ANTI-ULCER POTENTIALS OF THE STEM BARK EXTRACT OF *BRIDELIA FERRUGINEA*, BENTH.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE MASTER OF PHARMACY (M.PHARM) DEGREE OF THE UNIVERSITY OF NIGERIA NSUKKA.

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CERTIFICATION

The work embodied in this dissertation titled "Studies on the anti-ulcer potentials of the stem bark extracts of *Bridelia ferruginea*, Benth (Eurphobiaceae)" is an original investigation of the candidate, Nnamani, Marcellus Ejike PG/02/33244 and has not been submitted in part or full for any degree or diploma of this University and or other University. The work is hereby certified as meeting the requirements for the award of Master of Pharmacy (M. Pharm.) degree in Pharmacology and Toxicology.

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(Supervisor)

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DEDICATION.

To the memory of my late father, Mr. Pius Ugwoke Nnamani.

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I am most grateful to God Almighty for His blessings, guidance and protection throughout the period of this work. Glory be to Him now and forever.

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TABLE OF CONTENTS.

ABSTRACT.

The anti-ulcer activity of the methanol and petroleum ether stem bark extract of *Bridelia ferruginea*, Benth (Eurphobiaceae) was evaluated.

The preliminary phytochemical analysis of the methanol extract showed the presence of alkaloids, saponins, reducing sugars, tannins, carbohydrates, flavonoids, steroids, proteins, terpenoids and glycosides. The petroleum ether extract showed the presence of tannins, fats and oils, flavonoids, steroids and terpenoids.

The LD_{50} of the extracts was determined in mice by the technique of Lorke. The result revealed that the extracts at doses up to 5000mg/kg showed no signs of toxicity or death.

The anti-ulcer activities of the extracts were screened using gastric ulcers induced by absolute ethanol and indomethacin. The results obtained showed that both extracts are effective in inhibiting gastric lesions at doses of 200 mg/kg and above when compared with the inhibition of gastric ulcers with distilled water.

The extracts had no effect on the guinea pig ileum but inhibited the contractile effects produced by acetylcholine and histamine.

The extracts produced a concentration-dependent relaxation of the rhythmic contraction of rabbit jejunum. They also inhibited contractile responses induced by acetylcholine on the rabbit jejunum.

The results showed that stem bark of *Bridelia ferruginea* contain active ingredients with a therapeutic activity against gastric lesions.

CHAPTER ONE

1.0 INTRODUCTION

Ulcer is an open sore or lesion usually found on the skin or mucosal membranes of the body tissue. Peptic ulcer diseases (PUD) are disorders, which involve the occurrence of sores or lesions in the mucosal lining of the stomach (GU), pyloric channels (PCU), duodenum, (DU) and at or near the site of surgical anastomosis (post-operative ulcers). About 98% of peptic ulcers are either in the first portion of the duodenum or in the stomach in a ratio of about 4:1 (James and Vinay, 2003). Gastric ulcers (GU) and duodenal ulcers (DU) may be acute or chronic (Palmer and Penman, 1999).

There are different types of ulcers but the most common types are gastric ulcers and duodenal ulcers.

Gastric Ulcers (GU): This occurs when there are lesions in the gastric mucosal membrane of the stomach due to imbalance between impaired mucosal defensive and aggressive factors in the mucosal membrane of the stomach and duodenum. Though ulcers are not contagious or cancerous, gastric ulcers may become malignant.

Duodenal Ulcers (DU): This is located in the first 12 inches of small intestine beyond the stomach. This is mostly benign. Duodenal ulcers are more frequent in patients with alcoholic cirrhosis, chronic obstructive pulmonary diseases, chronic renal failure and hyperparathyroidism (James and Vinay, 2003).

Peptic ulcer associated with hyperacid secretion occurs mainly in patients with hypergastrinemia due to gastrinoma (McArthur et al, 1985). This condition occurs in Zollinger – Ellison syndrome. This syndrome is associated with abnormal increase in

basal acid secretion, which partly leads to the development of PUD. In this syndrome, the acid hyper secretion accounts for large load of acid in the small intestine, which causes diarrhea, malabsorption of fat and dilatation of small intestine. Another factor that can cause increase in basal acid secretion is hyperhistaminemia (McArthur et al, 1985). This occurs when there is systemic mast cell disease and basophilic leukemia. This condition is rare and can be managed with histamine – H_2 antagonists.

There may occur basal acid hyper secretion without hyper gastrinemia or other obvious cause. This condition is more common than Zollinger-Ellison syndrome (Soll et al, 1991). This is idiopathic acid hyper secretion and vagal hyperactivity is suspected to be the causative factor. This acid hyper secretion can be ameliorated by the use of low dose anticholinergics (Soll et al, 1991).

1.1 EPIDEMIOLOGY OF PEPTIC ULCER DISEASE:

Peptic ulcer was considered to be a disease of the young and middle – aged adults but it affects all age groups even children. The incidence of peptic ulcer is approximately 10% (Willemijntje and Pankaj, 2006) and it is also estimated that 50% of healthy individuals experience symptoms of peptic ulcer on daily basis (Willemijntje and Pankaj, 2006).

Incidence of PUD peaks after the fifth decade of life. Despite the advances in the diagnosis and treatment of the PUD, hospitalization for PUD has not declined. However, there is apparent decrease in DU in men (John, 1989). There is also marginal decrease in the complications like perforation and bleeding. The major complications of DU are bleeding, perforation, gastric outlet obstruction and penetration into the neighboring organs like the pancreas. Bleeding is the common cause of death from PUD especially in

geriatric patients. This is because this group of patient may have other associated disease condition. Mortality rate has decreased in both sexes (John, 1989).

The major environmental factors that are important in the pathogenesis of peptic ulcer are smoking and use of non-steroidal anti-inflammatory drugs (NSAIDs). Smokers have higher incidence of duodenal ulcer and higher rate of relapse due to slow rate of healing (Mc Guigan, 1991).

Mortality rate for smokers with ulcers is higher than non-smokers (Mc Guigan, 1991). Bleeding complication is higher in patients on NSAIDs than those not on NSAID (Soll et al, 1991). The incidence is also higher in those at risk of exposure to free radicals (Feldman et al, 1980). PUD is common in patients with some disease conditions such as chronic obstructive pulmonary disease and cirrhosis (John, 1989). The association of peptic ulcer and chronic obstructive pulmonary disease is not only due to smoking and chronic hypercapnia but also due to deficiency of alpha1 antitrypsin in both patients (John, 1989).

In cirrhotic patients with portocaval anastomosis, there may be increased acid secretion, sensitivity to gastrin and frequency of ulcer (John, 1989).

1.2 THE PATHOPHYSIOLOGY OF PUD:

There are several factors associated with the development of PUD. The pathogenesis of PUD essentially involves an imbalance between the aggressive factors (acid, pepsin, helicobacter pylori (HP), and bile salts) and defensive factors [mucin secretion, cellular mucus, bicarbonate secretion, mucosal blood flow and cell turnover] (Goel and Bhattcharya, 1991, Akah et al, 1998, Robert, 1981).

Ulcers are the sequale of the inability of the gastric or duodenal mucosal linings to resist the corrosive effect of acid on its surface. Therefore, the abnormality in acid secretion may not be the primary disorder but impairment of the defensive mechanisms (Bigheti et al, 2005). Studies have shown that acid secretion is either normal or below normal in gastric ulcer patients and that 40-70% cases of duodenal ulcer patients show acidity within normal range (Gupta et al, 1980) suggesting that other factors are also involved in ulcerogenesis. External factors, which may contribute to the formation of ulcers include infection with Helicobacter pylori, NSAIDs, free radicals, alcohols, smoking and of less important, stress. The periodic formation and healing of ulcers can be explained by the periodic changes in both the aggressive and defensive factors.

ACID SECRETION:

The stomach produces hydrochloric acid (HCl), pepsin (enzyme), intrinsic factors, bicarbonate and mucus. These secretions are required for the digestion of protein, absorption of vitamin B12 (cobalamin) and iron in the intestine and protection of the gastric mucosa from injury.

The gastric acid acts also as a disinfectant against ingested organisms as well as aid in iron absorption. The two major functional parts of the stomach are the proximal and the distal regions. The proximal parts constitute the oxyntic (parietal) cells and the peptic cells. These parts have secretory activities (John, 1989). The oxyntic cells are responsible for the secretion of acid and intrinsic factors while the peptic cells secrete pepsinogen (Arthur and John, 2000). The distal or pyloric region secretes hormones: gastrin and somatostatin. Somatostatin inhibits acid and pepsin secretion in the stomach

by both endocrine and paracrine routes (Rodger, 1992). In a negative feed back mechanism, gastrin secretion is inhibited by gastric acid in the antrum acting locally through somatostatin (Willemijntje and Pankaj, 2006).

The mucus neck cells, which are located in the entire regions of the stomach, are involved in the secretion of mucus and pepsinogen. The surface epithelial cells secrete bicarbonate, mucus, and pepsinogen.

Some factors influence gastric secretion such as time of day, food, and psychological states as well as other metabolic activities of the body (Russel, 1993). Therefore, there are three major phases of acid secretion; cephalic, gastric and intestinal (Russel, 1993).

There is a diurnal pattern of secretion with the highest and lowest secretion occurring respectively in the evening and early morning (Russel, 1993).

The rate of secretion is also increased during the cephalic phase (thought and sight of food) (Bigheti et al 2005). Gastric phase of secretion increases with the presence of protein in the gastric lumen (Arthur and John, 2000).

The enteric nervous system of the stomach produces some neuro- peptides that regulate gastric acid secretion, blood flow and motility. These peptides are gastrin releasing peptide, vasoactive intestinal peptide (VIP), leucine and methionine, enkephalins, substance P, calcitonin gene-related peptide, neuropeptide Y, and galanin (Ibu et al, 1994, John, 1989). Autonomic nervous system also regulates gastric function through the parasympathetic and sympathetic nerves supplying the stomach (Arthur and John, 2000, John, 1989).

Gastric function is also regulated by substances that are produced by cells in the gastric walls, which influence neighboring cells by local diffusion. This type of regulation called

paracrine regulation is exemplified by the stimulation of the parietal cells to secrete acid by locally released histamine (Walsh, 1992).

Parietal cell has secretaroy canaliculi lined with the enzyme; hydrogen-potassium ATPase [H^+ K^+ ATPase], responsible for acid secretion. Hydrolysis of ATP generates energy used by the enzyme to pump hydrogen ions into the secretory canaliculi in exchange for potassium ions (John, 1989).

Equimolar amount of chloride ions enter the canaliculi through nearby chloride channels, producing hydrochloric acid.

Other channels allow potassium ions to return to the canaliculi where they are exchanged for more hydrogen ions. From the canaliculi the hydrochloric acid goes into the stomach lumen. The secretion of acid into the lumen is proportional to the alkaline tide where bicarbonate is secreted from the base of the gastric glands to the area just below the surface epithelial cells (John, 1989). This flow of bicarbonate offers a protective mechanism for the gastric surface cells against the back diffusion of acid (John, 1989).

The action of hydrogen-potassium ATPase and carbonic anhydrase in the parietal cell generate hydrogen and bicarbonate ions from water and carbon dioxide (Arthur and John, 2000).

Parietal cell has three receptors, histamine H_2 receptor, gastrin receptor G, muscarinic cholinergic M_1 receptors that mediate the stimulation of acid secretion by the transmitters; histamine, gastrin and acetylcholine. The parietal cell acid secretion is stimulated by cyclic adenosine monophosphate (cAMP) and intra cellular Ca^{2+}. Hypercalcemia stimulates gastrin production, which in turn increases acid secretion (James, 2003). Gastrin analogue, pentagastrin, has a beta-alanine substitute acts on the

cck-2 gastrin receptor (Schubert, 2000) thereby increasing acid secretion and also stimulate histamine secretion of enterochromaffin cells (Angus and Black, 1982, Anderson et al, 1996). Histamine stimulates cAMP formation while acetylcholine and gastrin stimulate the increase in intracellular calcium ions (John, 1989). The activation of the muscarinic receptors in the parietal cell initiates sequential events triggered by G-protein leading to the liberation from the membrane phosphplipid of inositol triphosphate and diaceylglycerol (Clapham, 1995). The diacylglycerol activates protein kinase C, which increases gastric acid secretion while inositol triphosphate increases intracellular release of calcium and chloridic acid secretion (Clapham, 1995). Anticholinergics decrease parietal cell responses to histamine but the effect of anticholinergics in human beings is not as great as that of histamine H_2- receptor antagonists.

Parietal cell also has inhibitory receptors for prostaglandins apart from the stimulatory receptors for histamine (H_2), Gastrin (G) and muscarinic (M1) (Feldman et al 1980). There is increased maximal acid secretion in duodenal ulcers unlike in gastric ulcers. Gastric ulcer patients have almost the same maximal acid secretion as normal person (Aoygi and Sommerskill, 1996). Maximal acid secretion is a function of the parietal cell mass. There are more parietal cells in duodenal ulcer patients than in gastric ulcer patients (Ethrington et al, 1970). Disorders of the system, which may be responsible for the development of ulcer, occur when there are hyper secretion of acid and the concomitant *Helicobacter pylori* infection (Barocelli et al, 1997, Pearson et al, 1980).

PEPSIN

This is a proteolytic enzyme secreted in the stomach, which is involved in the digestion of proteins. There are two types of pepsin proenzymes, Group I and Group II pepsinogen (Ethrington et al, 1970). The Group I pepsinogens are produced by chief cells closely linked to parietal cells. Group II pepsinogens are secreted by the surface epithelial cells and by mucous neck cells located throughout the stomach and in the duodenum. Secretion of pepsinogen is stimulated directly by cholecytokinin and indirectly by gastrin (John, 1989). Histamine does not affect pepsinogen secretion. Cholecytokinin and secretin inhibit acid secretion. Cholinergic activation stimulates pepsin secretion more than acid secretion. Patient with PUD who has gastrinoma produces excess pepsin since pepsin secretion parallels acid secretion (John, 1989).

Though pepsin activity is implicated in the development of PUD in acidified environment, use of pepsin antagonist for the treatment of PUD has not been producing reliable result. Therefore, the exact mechanism of pepsin in the pathogenesis of PUD is yet to be elucidated.

MUCUS This is a glycoprotein sheet that covers the gastric mucosa. The layer consists of a semi solid gel. Mucus acts as a lubricant layer between the gastric mucosa and the gastric contents. It also acts as a barrier against acid. Mucus secretion is stimulated by cholinergic activation, prostaglandins and reflexes induced by the irritation of the gastric mucosa (Pearson et al, 1980).

HELICOBACTER PYLORI [HP] The organism was first documented to cause injury to the stomach in 1983 by two researchers in Australia (Walsh and Peterson, 1995). This

bacterium is implicated in gastritis and PUD. It is a spiral-shaped Gram-negative rod that can colonize epithelial cells lining the antrum of the stomach (Parsonnet et al, 1991). *Helicobacter pylori* cause chronic gastritis, plays an important etiologic role in the development of PUD and it is considered a risk factor in the development of gastric malignancies such as gastric mucosa-associated lymphoid tissue lymphomas and gastric adenocarcinoma (Parsonnet et al, 1994, Walsh and Peterson, 1995). It is well known that *Helicobacter pylori* is associated with alterations in the gastric epithelial cell cycle and apoptosis, higher levels of mononuclear and neutrophilic infiltrates, more severe atrophy and intestinal metaplasia (Antonio and Gaetano, 2004). Cell cycle alterations induce mitogenic signals and proto-oncogene expression that may trigger the development of cancer (Antonio and Gaetano, 2004). Though it does not grow in a very low P^H medium, it has mechanism of protecting itself from the acid of the stomach (Palmer and Penman, 1999). It produces urease, which metabolizes urea to generate energy and ammonia (Palmer and Penman, 1999). Other toxic products from the metabolism of urea by urease include ammonium chloride and monochloramine (James, 2003).

The ammonia enables it to survive in acidic medium of the stomach. The bacterium provokes a local inflammatory response in the underlying epithelium due to release of a range of cytotoxins, vacuolating cytotoxin (VaCA), cytotoxin associated gene (CagA), adhesins phospholipases and porins (Palmer and Penman, 1999, Lee, 1998). *Helicobacter pylori* cause increased production of proinflamatory cytokines such as interleukin (IL-1, IL-6 and IL-8) and tumor necrosis factor (TNF) (Dundon et al, 2001).

The infection of the antrum leads to the depletion of antral somatostatin and increased gastrin release from G cells (Willemijnte and Pankaj, 2006). Then, the gastrin stimulates G-receptors of the parietal cells leading to increased acid secretion. The increased acid secretion further damages the duodenal mucosa. Persistent damage of the duodenal mucosa stimulates the development of patches of gastric metaplasia in the duodenum, which in turn are colonised by *Helicobacter pylori* (Palmer and Penman, 1999; Dundon et al, 2001).

The colonization of the duodenum by HP allows more damage and eventual ulceration. While some HP infected patients develop PUD some do not. This variation may be due to the host factors and or bacterial factors (Davies et al, 1994). The host factors may involve the patient whose stomach linings have problems. PUD may have something to do with the combination of HP infection and the level of acid in the stomach (Davies et al, 1994). Some strains of *Helicobacter pylori* that produce vacuolating toxin (VacA) and cytotoxin associated geneA (CagA) cause more intense tissue inflammation and cytokine production (Israel et al, 2001). While cytotoxin (VacA) and cytotoxin associated gene A (CagA) occurs frequently in ulcers CagA only occurs in cancers (Parsonnet, 1996).

Cytotoxin associated geneA (CagA) is a powerful stimulus for the production of interleukin-8 (IL-8) by the epithelial cells (Israel et al, 2001).

Helicobacter pylori also cause inability to absorb vitamin B12 in some individuals (Parsonnet, 1996).

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

These are used to treat long term painful conditions like arthritis. They include such agents as ibuprofen, indomethacin, aspirin and diclofenac. These agents when used in high daily doses for a long time cause ulcers (Ivey, 1988).

They cause mucosal damage by inhibiting cycloxygenase activity thereby reducing the formation of protective prostaglandins (E_1&E_2). This inhibition interferes with the protective mechanisms such as mucus and bicarbonate secretion, surface epithelial hydrophobicity and mucosal blood flow (Langman et al, 1991, Soll et al, 1991).

These changes promote back diffusion of acids through the breached surfaces to destroy cells, capillaries and veins causing haemorrhagic ulcer. NSAIDs also enhance leukotriene synthesis resulting in damaging effects. Moreover, mucosal ATP synthesis and cell turnover processes are reduced by NSAIDs. These changes by NSAIDs can induce gastric damage through the generation of reactive oxygen species [ROS] (Vaananann et al, 1991; Yoshikawa et al, 1993).

NSAIDs in addition block gastric peroxidase enzymes and increase mucosal hydrogen peroxide and hydroxyl (OH^-) level to cause oxidative mucosal damage (Banerjee, 1990). This (OH^-) causes lipid peroxidation and increased gastric lesions induced by NSAIDs (Pihan et al, 1987). This lipid peroxidation causes decrease in the level of glutathione (GSH) in the gastric mucosa. Glutathione is a free radical scavenger.

There are some risk factors for NSAIDs – induced ulcers and they are as follows:

1. Past history of PUD
2. Past history of adverse event with NSAIDs
3. Concomitant use of corticosteroid

4. High or multiple NSAIDs.

5. Individual NSAIDs. Some are higher than others in GI toxicity. Azapropazone and piroxicam are highest compared with ibuprofen.

6. Age. The risk is high at above 60 years.

SMOKING:

Smoking relaxes the pyloric sphincter, which permits the regurgitation of bile into the stomach, which damages the gastric and duodenal mucosa (Mc Guigan, 1991). This increases the risk of getting ulcers because the nicotine in cigarettes stimulates acid secretion in the stomach (Arthur and John, 2000). This risk is higher for gastric ulcer than duodenal ulcer. Smoking delays ulcers healing (John, 1989).

ALCOHOL

Overtime, alcohol wears down the linings of the stomach and intestines. Hence, it is a predisposing cause of acute and haemorrhagic gastric erosion in humans (Debashis et al, 2002). Ethanol lowers the concentration of non-protein sulphydryls especially glutathione (Szabo et al, 1981) thereby exerting ulcerogenic effect by increasing reactive oxygen specie formation (Pihan et al, 1987, Szelenzyl et al, 1985).

The development or prevention of vascular injury in the gastric mucosa plays a crucial role in gastric mucosal injury and protection (Szabo et al, 1990. Cho et al, 1992).

Haemorrhagic mucosal lesions due to ethanol, hydrochloric acid and sodium hydroxide (NaOH) in rats are preceded by micro vascular damage, increased vascular permeability

and capillary stasis (Szabo et al, 1986). Leukorienes are one of the important causes of ulceration induced by ethanol (Goel, 2002).

FREE RADICALS

Free radicals and lipid peroxidation may contribute to the formation of gastric lesions (Itoh et al, 1985, Smith et al, 1987, Gutteridge, 1995).

Free radicals also play some roles in the development of gastric mucosal lesions induced by alcohol (Salium, 1990). These radicals through the release of lysosomal enzymes from cellular membranes worsen tissue damage (Pal, 1994).

Studies have shown that oxidative stress is also involved in the pathogenesis of peptic ulcers (Bairy et al, 2002). Reactive Oxygen specie generated in the cells of aerobically respiring organisms due to many factors have been implicated in the pathogenesis of many human sufferings like Parkinson's, Alzheimer's, Huntington's diseases, liver cirrhosis, ulcer, artherosclerosis and cancer (Ajaikumar et al, 2005). Reactive oxygen specie (ROS) is implicated in certain ischaemic, cardiovascular and pulmonary diseases, cataratogenesis and reproductive disorders (Halliwell et al, 1989).

The involvement of reactive oxygen specie in the pathogenesis of gastric ulceration was first evident from the studies on ischemia reoxygenation – induced gastric muscosal injury (Yoshikawa et al, 1989, Yuda, 1993, Perry et al, 1996).

There are experimental and clinical evidence, which suggest that mucosal damage by ethanol, NSAIDs, and Helicobacter pylori is mediated through reactive oxygen specie (Davies et al, 1994, Vaananann et al, 1991, Yoshikawa et al, 1993, Pihan et al, 1987, Szelenzyl et al, 1985, Yoshikawa et al, 1996, Phull et al, 1995).

Reactive oxygen species may play an important role in gastric ulceration induced by several types of stress (Yoshikawa et al, 1996, Phull et al, 1995).

Reactive oxygen specie also decreases the levels of endogenous antioxidants such as glutathione (GSH), alpha-tocopherol and ascorbate and make the mucosa more prone to oxidative damage (Phull et al, 1995).

STRESS

Stress is also a major cause of gastric ulcer (Barocelli et al, 1997). Stress as implicated in the development of ulcer consists of physical and emotional stresses.

Emotional stress may make an ulcer more painful and more difficult to heal but the stress itself does not cause an ulcer (Cho et al, 1992). Emotional stimuli frequently increase interdigestive gastric secretion to 50ml or more [and highly peptic and acidic] per hour in very much the same manner that the cephalic phase of gastric secretion at the onset of a meal (Arthur and John, 2000).

Mucins are high molecular weight glycoprotein responsible for the gel forming property of gastric mucus secretion (Allen et al, 1993).

Stress reduces the quality and amount of mucus adhering to the gastric mucosa (Allen et al, 1993).

In condition of emotional stress, there are greater destruction of mucus and a decreased synthesis of its components as well as a change in quality thereby affecting the biochemical processes of translation, acylation and glycosylation of the ribosomal peptides (Tsukada et al, 1989).

1.3 DIAGNOSIS OF PUD

Peptic ulcer disease can be diagnosed by some symptoms and signs experienced by individual patients. Other ways of diagnosis include physical examination, laboratory studies and methods of visualization of ulcers like endoscopy.

1.4 SYMPTOMS AND SIGNS

Gastric ulcer and duodenal ulcer share common symptoms and signs, which include the followings:

PAIN: This is the most common ulcer symptom. It is a gnawing or burning pain in the upper quadrant of the abdomen or in the back.

In the abdomen it is between the breastbone and the navel. The gastric ulcer pain can be distinguished from the duodenal ulcer pains by its persistence after food as well as its continuous rather than episodic in nature (Walsh, 1992). DU is associated with heartburn while gastric ulcer does not produce heartburn.

BLEEDING: Bleeding ulcers can be painless and may present as haematemesis, melena, anaemia and hematochezia (Walsh, 1992).

VOMITING: Patients with gastric obstruction present with repeated vomiting leading to dehydration and chloride depletion (Walsh, 1992).

DYSPEPSIA: Dyspepsia as a symptom may indicate any one of several disease states such as Helicobacter pylori infection, peptic ulcer disease, gastroesophageal reflux disease (GERD), gastric motility disorders, non-ulcer dyspepsia and malignancy (Walsh,

1992). It is a common complaint with the estimate of 4-5% of patients naming dyspepsia as the primary reason for visiting their primary health care provider (Review, 1998).

The appropriate diagnostic approach for dyspepsia is endoscopy, which shows about 60% of patients with dyspepsia not having abnormal findings or definitive etiology (functional or non-ulcer dyspepsia) (Review, 1998).

Dyspepsia is a relapsing condition with 50-80% rate of relapse. This requires maintenance therapy in many cases (Devauli et al, 1999). Other symptoms are nausea, anorexia and weight loss.

1.5 LABORATORY STUDIES

The laboratory studies that are required in the investigation of peptic ulcer disease are the invasive and non-invasive tests for Helicobacter pylori (Nomura et al, 1991).

The non-invasive method involves serological and urea breathe test. The invasive methods include histology rapid urease test and microbiological culture (Nomura et al, 1991). Invasive method involves the use of an endoscopy to obtain biopsy specimen for the evaluation while non-invasive method depend on blood and breathe samples (Radeneck and Graham, 1997). Blood tests measure anti-bodies to make a diagnosis while the breath test uses radioactive or non-radioactive forms of urea, which the patients drink (Radeneck and Graham, 1997).

1.6 VISUALIZATION OF ULCERS

There are techniques for the visualization of ulcers, which include:

i) **ENDOSOCOPY:** This involves the use of an endoscope which is guided into the throat and down into the esophagus and finally into the stomach and upper intestines. After successful introduction of the instruments, the inner lining of these organs can be observed from the camera on a television screen. During endoscopic technique, tissues can be taken and tested for HP. This technique has high specificity and sensitivity for PUD. It also offers an opportunity for biopsy and histological examination of questionable lesions. The major limitation of this technique is the high cost, which very few can afford. It is indicated for patients older than 45 years especially with alarm symptoms (American Society for Gastrointestinal Endoscopy, 1999).

ii) **RADIOGRAPHY:** This method is less accurate than endoscopy for the diagnosis of small ulcers. This involves the radiological study of the stomach, duodenum and esophagus. This is carried out by administering a barium meal to the patient and obtaining the X-ray of the gastrointestinal tract. The presence of ulcer will be outlined on the X-ray.

1.7 COMPLICATIONS OF PUD

Complications of peptic ulcer disease include bleeding, gastric outlet obstruction, perforation and penetration.

1.8 TREATMENT OF PUD

1.8.1 GOALS OF THERAPY

The goals of treatment of PUD are the following:

i) Relief of pain

ii) Healing

iii) Prevention of relapses

iv) Prevention of complication

1.8.2 NON-SPECIFIC THERAPEUTIC MEASURES

This approach plays adjunctive roles in the treatment and management of PUD. The following measures are involved in this approach.

Avoidance of Smoking: Smoking delays ulcer healing and increases recurrence rate. Hence, avoidance of smoking will have beneficial effects on healing and recurrences (Palmer and Penman, 1999).

Stress Management: Since stress especially emotional stress worsens ulcer, its management will facilitate healing (Cho et al, 1992). Therefore, exercise plays some roles in reducing stress. Exercise relieves and promotes weight loss, muscle gain and feeling of well-being.

It also releases hormones, endorphins, which not only relieve stress but also cause a reduction in cortisol levels (Walsh, 1992).

Restriction of Alcohol Consumption: This is important in patients with alcohol cirrhosis as it affects healing as well as increases the rate of recurrences after initial healing (Palmer and Penman, 1999).

1.8.3 SPECIFIC THERAPEUTIC APPROACHES FOR PUD

Acid suppressive therapy has marginal benefits over placebo as evidenced by a meta analysis of antisecretory agent for non-ulcer dyspepsia showing a benefit of only 20% over placebo (Dobrilla et al,1989).

Agents used for the treatment of PUD have limiting side effects (Barrowwan et al, 1992). There is no single agent with absolute healing activity without relapse (David, 1998).

ANTACIDS

The only relief from the pain of ulcers has been provided for decades by the neutralization of gastric acid with antacids. Antacids by their alkaline nature act by neutralizing the gastric acid. This neutralization reaction weakens the corrosive effects of the acids and reduces ulcer pains. They also strengthen the mucosal defensive mechanism through their stimulation of prostaglandin production in the mucosa (Mc Quaid, 2004). Moreover, neutralization reduces the peptic activity in the gastric juice and inactivates pepsin at pH above 5 (Arthur and John, 2000).

Animal studies have demonstrated mucosal protection by antacids either through the stimulation of prostaglandins production or the binding of an unidentified injurious substance (David, 1998).

Antacids affect bowel movement and secretions. Aluminum containing antacids possess constipating effect by decreasing bowel motility while magnesium-containing antacids have cathartic effects by increasing bowel motility (D'Arcy, 1987). Antacids containing mixtures of aluminum and magnesium compounds do not significantly change bowel function (D'Arcy, 1987).

CYTOPROTECTIVE AGENTS:

The therapeutic approaches aimed at reducing gastric acid secretion and therapeutic measures involving the use of a variety of cytoprotective agents are also utilized in the treatment of peptic ulcer disease. These cytoprotective agents strengthen the gastric and duodenal defenses (Venkataanganna et al, 1998). These compounds include bismuth subsalicylate and colloidal bismuth subcitrate.

Another important agent is sucralfate, which is a product of the reaction between sucrose octasulfate and aluminum hydroxide. At acidic P^H, it undergoes polymerization and cross linking of sucralfate to form a gel that is viscid and demulcent (McCarthy, 1999).

The mechanism of cytoprotective and healing properties of sucralfate involves stimulation of prostaglandin synthesis, adsorption of pepsin and stimulation of local production of epidermal growth factor (McCarthy, 1999).

Food or antacids do not affect the gel's adherence integrity. The gel adsorbs proteins in food thereby increasing the cytoprotective layer (McCarthy, 1999).

PROSTAGLANDIN ANALOGS.

Prostaglandin analogs are useful in the treatment of peptic ulcers.

Gastric mucosa synthesizes prostaglandins especially PGE_2 and PGI_2. These prostaglandins inhibit the secretion of acid and stimulate the secretion of mucus and bicarbonate as well as blood flow (Ibu et al, 1994). Misoprostol, a prostaglandin E_1 analog, is effective in the treatment of PUD (Collins, 1990). It is particularly useful for patients who require NSAIDs for the treatment of arthritis and other diseases and for the

prevention of gastric ulcers induced by NSAIDs (Collins, 1990). PGE_2 protects the stomach against erosive actions of gastric acids, pepsin, NSAIDs and alcohol (Pennington, 1985).

HISTAMINE H_2 – RECEPTOR ANTAGONISTS

The development of histamine H_2 – receptor antagonists not only provided specific class of antisecretory agents but also revolutionized PUD management and treatment.

These agents do not have any effect on histamine H_1 – receptors. Their development in the seventies revolutionized the treatment of PUD. Drugs in this class include cimetidine, ranitidine, famotidine and nizatidine. This group of drugs is more hydrophilic than H_1 blockers thereby making them less penetrable to the CNS.

H_2 – histamine receptor antagonists competitively inhibit the interaction of histamine with H_2 – receptors thereby blocking gastric secretion by histamine and other H_2 agonists in a dose dependent manner. This antagonistic effect of these agents is most pronounced in gastric acid secretion (Bertaccini et al, 1982). Study has shown that delay in gastric emptying will slow the evacuation of the gastric content which in turn enhances the absorption of orally administered anti ulcer agents and eventual promotion of healing (Bertaccini et al. 1981).

Some H_2 blockers were shown to reduce gastric emptying in rats by a mechanism totally independent of H_2 – receptor blockade (Bertaccini et al, 1982).

The physiological effects of H_2 blockers on H_2 receptors in vascular and bronchial smooth muscles are not clinically significant (Mohammed et al, 1994). They inhibit gastrin and muscarinic agonist – stimulated acid secretion.

These agents inhibit basal and nocturnal acid secretions. They alter the cephalic, gastric and intestinal phases of acid secretion (Russel, 1993).

They also reduce the volume of gastric juice and its hydrogen (H^+) concentration as well as the output of pepsin and intrinsic factor (Binder et al, 1978). H_2 – blockers and other antisecretory drugs are effective in PUD and hypersecretory states associated with mastocytosis where acid hyper secretory activities are involved (Bambery et al, 1992; Mohammed et al, 1994).

ERADICATION OF HELICOBACTHER PYLORI

The recognition of the role of Helicobacter pylori in causing gastritis and in the development of PUD provided the therapeutic insight that the eradication of this small, spiral-shaped, flagellated gram-negative bacterium would be a useful strategy for promoting the healing of ulcers and prevention of their recurrence.

Single agent therapy for HP infections has proven relatively ineffective in vivo and has led to the emergence of resistant strains. Multiple drugs are needed in the eradication of HP infection because of resistance development. Evidence has shown that probiotics, which are live microbial food supplements beneficially, affect the host by improving its microbial balance (Fuller, 1991). Studies have also shown that ingesting lactic acid bacteria exerts a suppressive effect on HP infection in both humans and animals while supplementing with *Lactobacillus* and *Bifidobacterium*- containing yoghurt (AB-yoghurt)

was shown to improve the rates of eradication of HP in humans (Kuan-Yuan et al, 2004). The inhibition of HP growth occurred as a result of in vitro production of organic acid by *Lactobacillus acidophilus* (Midolo et al, 1995). *Lactobacillus acidophilus* supernatant decreased HP viability in vitro and decreased urease activity and the histopathologic degree of gastric lesions in mice infected with Helicobacter felis (Coconnier et al, 1998). Elimination of HP infection leads to a significant increase in the levels of the powerful appetite-stimulating hormone, ghrelin in the tissues of the stomach where it is produced (Tatsuguchi et al, 2004). This may be responsible for the increase in weight of patients undergoing treatment for the eradication of HP (Murray et al, 2003 and Baena et al, 2002). Study has shown that modest consumption of wine and beer (approximately 7 units/week) protects against HP infection, presumably by facilitating eradication of the organism (Liam et al, 2002).

Helicobacter pylori peptic ulcers are treated with drugs to kill the bacteria, drugs to reduce stomach acid and drugs to protect the lining of the stomach (Wash and Peterson, 1995). This triple therapy has been shown to kill the bacteria, reduce ulcer symptoms and prevent recurrence in over 90% of patients (Wash and Peterson, 1995). The antibiotics most commonly used to kill the bacteria are amoxicillin, Claritromycin, metronidazole and tetracycline. The drugs to reduce stomach acids are the histamine (H_2) blockers (cimetidine. famotidine, nizatidine and ranitidine) and proton pump inhibitors (lansoprazole and omeprazole). The drug that is used to protect stomach lining is bismuth subsalicylate or bismuth subcitrate.

The major draws back for this multiple therapy are compliance, cost and unpleasant side effects.

PROTON PUMP INHIBITORS (PPIs)

The inhibitors of the enzyme, H^+K^+-ATPase, which is responsible for the final step in the acid secretion by the parietal cells, have offered effective means of selectively blocking the proton pump (Lindberg et al, 1990). The enzyme, H^+K^+-ATPase (proton pump) mediates acid secretion in parietal cells. Inhibitors of the enzyme play remarkable role in the inhibition of acid secretion (McTavish et al, 1991). The inhibitors include substituted benzimidazoles (Lansoprazole and Omeprazole). A prototype, Omeprazole is P^H sensitive and at neutral P^H it is lipid soluble, chemically stable and weak base (Barradell et al, 1992).

At neutral P^H, the drugs are diffused into the secretory canaliculi and become protonated and trapped (McTavish et al, 1991). The protonation is associated with the structural rearrangement of the molecules to form active metabolite, sulfenic acid and a sulfonamide (McTavish et al, 1991).

The activated forms covalently binds to the sulfhydryl groups of the H^+K^+-ATPase in such a manner that requires two molecules per molecule of enzyme.

PPIs have specific activity due to their selective effect on H^+K^+-ATPase, their acidic requirement for the generation of active metabolite, trapping within the acidic canaliculi of the protonated drugs and sulfenamide.

Due to covalent binding of the active metabolite, acid secretion can only resume with the synthesis of the H^+K^+-ATPase (Barradell et al, 1992).

Studies have shown that lansoprazole has similar mechanism of action but its inhibitory effect can be reversed by a mechanism requiring glutathione and that synthesis of new enzyme is not required for the resumption of acid secretion (Barradell et al, 1992).

They do not affect gastric motility and have marginal changes in the amount of gastric juice, pepsin and intrinsic factor. Their actions persist even after the withdrawal of the drug. They are available as sustained release capsules. They are formulated into micro encapsulation because of ease of degradation by gastric acid.

1.8.4 HERBAL AND NATURAL PRODUCTS FOR THE TREATMENT OF PUD

Reports on clinical evaluation of conventional anti-ulcerogenic drugs show that there are incidences of relapses, adverse effects and danger of drug interactions during ulcer therapy (Goel and Sairam, 2002). As a result, the search for an ideal anti-ulcer drug continues and has also been extended to herbal drugs in search for new and novel molecules which afford better protection and decrease the incidence of relapse (Goel and Sairam, 2002).

Herbal medicines play an important role in health care delivery and about 70-80% of the population depends on traditional healers for most of their ailments including peptic ulcer (Akah et al, 1998). Diseases such as AIDS, herpes, malaria, tuberculosis and other emerging multi-drug resistant diseases continue to encourage research efforts into herbal medicines (Rabiu, 2002). Herbal drugs possess potential in combating various diseases conditions (Amos et al, 2001, Pezzuto, 1997). Although herbs are useful alternative remedies for some human ailments, some can be harmful (Adesina, 1998).

About 60% of the world's population relies almost entirely on plants for medications and natural products, which have long been recognized as an important source of therapeutically effective medicine (Ajaikumar et al, 2005). Six of the top twenty (20) pharmaceutical drugs sold in 1996 were natural products and more than 50% of this top twenty (20) was linked directly to natural product research (Balandrin et al, 1996). The high cost of newly available drugs for peptic ulcer disease is responsible for the persistence, morbidity and mortality of the disease in third world countries due to low per capita (Shayne, 2002). The prevalent rate is decreasing in developed but increasing in developing countries (Shayne, 2002). Many plants have anti ulcer constituents (Akah et al, 1996). Studies have shown that *Bacopa monniera* and *Azadirachta indica* have anti-ulcer and ulcer healing activities, which are attributed to their effects on various mucosal offensive and defensive factors (Drababu et al, 2004).

Since free radicals and *Helicobacter pylori* are implicated in the pathogenesis of PUD, natural products with antioxidant, antimicrobial as well as anti ulcer properties offer beneficial therapeutic outcome. As a result, research has shown that water extract of stinging nettle (*Urtica dioica* L) possesses antioxidant properties and anti ulcer activity against ethanol – induced ulcerogenesis (Ray, 2004).

There is extensive experimental evidence that indicates certain substances through scavenging of free radicals protect the gastric mucosa (Galvin and Szabo, 1992).

Shankha bhasma provides anti-ulcer activity in rats by acting as gastric cytoprotective agent and modulation of free radicals (Pandit et al, 2000).

Bacopa monniera extract exerts anti-ulcer activity through its anti *Helicobacter pylori*, increase in prostanoids (PGE and $PG1_2$), increase in mucin secretion, increase in life span of mucosal cells and gastric antioxidant effect (Ray, 2003).

Centella asiatica water extract and its active ingredient, asiaticoside are used as anti gastric ulcer agents due to their ability to reduce the size of ulcers and the concomitant attenuation of myeloperoxidase activity at the ulcer tissues (Sairam et al, 2001).

The methalol extract of *Punica granatum* (Pomegranate) possesses gastroprotective activity through its antioxidant mechanism (Ajaikumar et al, 2005).

Studies have also shown that "Parsley" *Petroselinum crispum* extract possesses antiulcerogenic activity by replenishing ethanol-induced depleted gastric wall mucus and non-protein sulfhydryl contents (Al-Howiriny, et al 2003).

Researchers have found that Guarana (*Paullinia cupana* Mart) has gastroprotective properties (Campos et al, 2003). Since urease of *Helicobacter pylori* is essential for its colonization, research (Matsubara et al, 2003) has focused on foodstuffs, which inhibit the activity of this enzyme. Among some plant – derived foodstuff sample tested, some tea and rosemary extracts were found to clearly inhibit *Helicobacter pylori* urease in vitro (Matsubara et al. 2003). This inhibition is attributed to the hydroxyl group of 5(1) – position of the active constituent, catechins (Matsubara et al, 2003).

Since the acquisition by *Helicobacter pylori* of resistance to antibiotics has become a serious problem, tea and tea catechins may be very safe resources to control *Helicobacter pylori* - associated gastroduodenal disease.

Whey protein concentrate protects gastric mucosa from ethanol damage and that the protection depends on sulfhydryl compounds present in the whey protein concentrate, including its capacity to stimulate glutathione synthesis (Rosaneli et al, 2002).

Furthermore, CO_2 – extracted sea buckthorn seed and pulp oils have both preventive and curative effects against experimental gastric ulcers in rats (Xing et al, 2002).

Ganoderma lucidum Polysaccharides from *Ganoderma lucidum* produced a mucosal healing effect in the rat model, perhaps due partly to the suppression of tumour necrosis factor (TNF – alpha) alpha (Gao et al, 2002).

Propolis extract exhibited dose–dependent superoxide scavenging activity and antioxidant effects on absolute ethanol – induced lipid peroxidation in rat gastric mucosal homogenates thereby protecting the gastric mucosa from oxidative stress (Ray, 2002).

Other plants with anti-ulcerogenic properties include unripe plantain banana (*Musa sapientum* var. *paradisiacal*), ginger (*Zingiber officinale*) and Satavari (*Asparagus racemosus*) [Goel, 2002].

Studies have equally shown that regular intake of yoghurt containing *Bifidobacterium* and *Lactobacillus* plays curative and healing roles in PUD by suppressing HP infection (Kuan-Yuan, 2004).

There are two types of mucins produced in the gastric mucosal layers. Mucins from the surface of the stomach support the growth of *Helicobacter pylori* while mucin from the deeper layers of the stomach lining (with O-glycans that have alpha1, 4-linked N-acetylglucosamine) inhibits the growth of *Helicobacter pylori* by blocking its biosynthesis of cholesteryl alpha-D-glucopyranoside which is a major cell wall component (Masatomo et al, 2004). Masatoma et al believe that the bug killing mucin

could help in the design of safer drugs that could treat stomach ulcers and prevent stomach cancer associated with *Helicobacter pylori* infections (Masatomo et al, 2004). They equally predicted that cows could be bred to produce inhibitory mucins in their milk, which will offer an inexpensive way to help eradicate *Helicobacter pylori* infection (Masatomo et al, 2004).

1. 9. 0 BRIDELIA FERRUGINEA.

CLASSIFICATION.

Kingdom .....Plantae.

Division.....Angiospermae.

Class.....Archichlamydeae.

Order .....Geraniales.

Family .....Euphorbiaceae.

Genus .....Bridelia.

Specie.....ferruginea.

1.9.1 PLANT DESCRIPTION

This is a shrub, which grows up to 18m high and may be 1.5m in width. The stem is often crooked with branches occurring at the lower regions. The bark is gray, rough and often scaly. It has elliptic leaves of 4-10cm in length and about 3.5cm in breath. The leaves possess wavy margins. It bears creamy-yellow, sweet scented flowers, which appear between February and August. Its fruits occur in July-September (Iwu, 1993).

1.9.2 ETHNO BOTANICAL INFORMATION.

In southwestern Nigeria, the stem bark is used as an antidote for arrow poison (Iwu, 1993). In the traditional Yoruba medicine-'Agbo Pot' which is used for paediatric illness has as its ingredients the leaves, roots and bark of *Bridelia ferruginea* (Iwu, 1993). The whole plant is used for the treatment of intestinal and bladder disorders and externally for skin infections and eruptions, and the leaves and stem barks are indicated for arthritis (Dalziel, 1948).

In Ezimo, Udenu Local Government Area, Enugu State, the fresh stem bark is used to arrest bleeding of fresh wound. Preparing paste of the fresh stem bark and placing it on the wound achieve this.

1.9.3 SCIENTIFIC REPORTS

Reports on the plant have shown that aqueous leaf extracts of the plant posses hypoglycemic activities (Addae-Mensah and Achenbach, 1985, Onunkwo et al, 1996).

Rutin, one of the active constituents of *Bridellia ferruginea* lowers blood sugar level of fasted rabbits (Addae-Mensah and Achenbach, 1985).

Furthermore, studies have shown that the aqueous extract of *Bridelia ferruginea* stem bark caused an inhibition of increase in vascular permeability in both cyclophosphamide – induced hemorrhagic cystitis and acetic acid-induced vascular permeability in rats and mices (Olajide et al, 2000). *Bridelia ferruginea* also produced stabilization of erythrocytes exposed to heat and stress-induced lysis (Olajide et al, 2000).

Studies further suggested that the extract of the stem bark has anti-inflammatory properties, which are attributable to its suppression of tumour necrosis factor alpha up-

regulation (Olajide et al, 2003). *Bridelia ferruginea* stem bark is used for the treatment of rheumatic pains in traditional medicine by inhibiting the xanthine oxidase as well as superoxide scavenging activity at micromolar concentration (Cimanga et al, 2001).

Its constituents responsible for these activities are 3-O-methylquercetin, myricetin, ferrugin and quercetin 3-O-glucoside (Cimanga et al, 2001). The stem bark and leaf extracts of *Bridelia ferruginea* have a contractile effects on the smooth muscle of the bladder (Onoruvwe et al, 2001). The extracts of *Bridelia ferruginea* have anti thrombotic effects (Olumayokum, 1999). A chemical examination of the ethylacetate leaf extract of *Bridelia ferruginea* showed the presence of two coumestanflavonoids, bridelilactone and bridelilactoside (Okunji, 1982). There are aesculetin and scopoletin as well as flavonoids from the petroleum spirit extract of the leaf (Okunji, 1982). Petrol extract and aqueous methanolic extracts yielded respectively terpenoids and flavonoid glycosides [quercetin -- 3 -- neohesperiaoside (rutin)] (Addae-Mensah et al, 1985).

1.10 OBJECTIVE.

Though there have been studies on *Bridelia ferruginea*, there is no reference in literature on the possible ant-ulcerogenic activity of this plant either in man or animal. This work was aimed at evaluating the antiulcer activity of petroleum-ether and methanol extracts of the stem bark of *Bridelia ferruginea* in ulcer induced experimental rats.

Fig: 1.1 The Plant, Bridelia feruginea
(The whole pant)

Fig: 1.2 The Plant, Bridelia feruginea
(The developing fruit)

Fig: 1.3 The Plant, Bridelia feruginea
(The leaves and the stem)

Fig: 1.3 The Plant, Bridelia feruginea
(The leaves and the stem)

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 PLANT MATERIALS

The plant materials, stem barks, were collected in May 2005 in Ezimo, Udenu Local Government Area of Enugu State in Eastern Nigeria. The plant was identified by Mr. Ozioko, O. of the Botany Department, Herbarium section, University of Nigeria, Nsukka [UNN]. A voucher specimen of the plant was kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The bark was separated from the stem, cleared of the dead outer parts and the thorns. It was air dried for over a week and milled into powdered form.

2.2 PREPARATION OF EXTRACT

The powdered bark of *Bridellia ferruginea* was extracted with petroleum ether (60-80 grades) in a soxhlet extractor for 4 hours. The extract was concentrated using a rotary evaporator. The residue was further extracted with absolute methanol in a soxhlet extractor for 5 hours. The extract was also concentrated in a rotary evaporator.

2.3 PHYTOCHEMICAL ANALYSIS:

The fresh methanol and petroleum ether extracts (ME & PE) were analysed for the presence of carbohydrate, tannins, glycosides, flavonoids, terpenes, steroids, resins, volatile oil, saponins, anthraquinones and alkaloids according to the methods of Evans (1989).

2.4.0 ANIMAL EXPERIMENTS

2.4.1 EXPERIMENTAL ANIMALS

Adult healthy animals of either sex were used. Albino mice [30.0-32.0 g], Guinea pigs [350.0-400.0 g] and New Zealand rabbits [1.5-3.0 kg] were used.

The mice and rats were obtained from the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. These animals were fed on standard pellets. The Guinea pigs and rabbits were obtained locally and maintained on guinea grass [*Panicum maximum*] and stabilized in the animal house for two weeks before being used for experiments. All the animals used had free access to clean water.

2.4.2 TOXICOLOGICAL STUDIES:

Adult albino mice of either sex (30.0-32.0 g) were used for these experiments. The LD_{50} was determined using the technique of Lorke (1983) and acute toxicity based on behavioral, autonomic and neurological profile were studied by following the Miller and Tainter (1944) techniques.

2.4.3 ACUTE TOXICITY STUDIES

The petroleum ether [PE] extract was prepared by preparing a suspension of 5% Tween 20 in distilled water while the methanol extract [ME] was prepared using distilled water. The doses of the drugs [extracts] used were administered intraperitoneally [I.P].

2.4.4 LD_{50} DETERMINATION:

The LD_{50} was determined by the method of Lorke (1983) using doses of 10.0 mg/kg, 100.0 mg/kg and 1000.0 mg/kg body weight administered to three groups of three mice each in the first phase of the investigation. The first phase aids the determination of the approximate range of acute toxicity and based on the result obtained from this phase, two or three doses were chosen for the second phase of the investigation. The animals were observed for 24 hours for signs of toxicity as well as death. The second phase involved the administration of doses of 1600.0 mg/kg, 2900.0 mg/kg and 5000.0mg/kg body weight. The number of animal in the second phase per group is one.

2.5.0 THE PHARMACOLOGICAL STUDIES

In vivo pharmacological studies were carried out on the crude methanol (ME) and petroleum ether (PE) extracts to evaluate their possible anti-ulcerrogenic activities.

2.5.1 DETERMINATION OF THE EFFECTIVE DOSE OF THE EXTRACTS.

The adult Wistar rats were starved of food for 24 hours prior to the experiment but allowed free access to water. The rats were randomized and divided into five groups of four animals each according to the treatment employed (Distilled water 5.0 ml/kg, cimetidine 100.0 mg/kg, ME 200.0 mg/kg, ME 400.0 mg/kg, and 800.0 mg/kg body weight) per oral

After 30 minutes of treatment, each animal received 1ml of absolute ethanol orally, according to the methods of Robert [1979]. After 1 hour, the animals were sacrificed by blow on the head and their stomach were removed and opened along the greater curvature then rinsed carefully under a running tap and pinned on a cork board and examined with a hand lens (x10). The number and severity of lesions were scored as below:

1..... less than or equal 1mm.

2..... 1-2mm.

3..... >2mm.

The ulcer index (U.I) was calculated by using the formula, U.I = 1x [No. of lesions of grade 1] + 2x [No. of lesions of grade 2] + 3x [No. of lesions of grade 3]. The overall score is divided by a factor 10 (Main and Whitle, 1975). Mean score for each group was calculated and expressed as ulcer index. Percentage ulcer protection was calculated as follows (Suziki et al, 1976).

1- [Ulcer index with extract/ulcer index with Distilled water] x 100.

The procedure was repeated using varying doses of PE (200.0 mg/kg, 400.0 mg/kg and 800.0 mg/kg).

2.5.2 ABSOLUTE ETHANOL – INDUCED GASTRIC LESIONS:

The adult Wistar rats were starved of food for 24 hours prior to the experiment but allowed free access to water. The rats were randomized and divided into three groups of four animals each according to the treatment employed [Distilled water 5.0 ml/kg, cimetidine 100.0 mg/kg, and ME 200.0 mg/kg body weight]

After 30 minutes of treatment, each animal received 1ml of absolute ethanol orally, according to the methods of Robert (1979]. After 1 hour, the animals were sacrificed by blow on the head and their stomach were removed and opened along the greater curvature then rinsed carefully under a running tap and pinned on a cork board and examined with a hand lens (x10). The number and severity of lesions were scored as below:

1..... less than or equal 1mm.

2..... 1-2mm.

3..... >2mm.

The ulcer (U.I) is calculated by using the formula, U.I = 1x [No. of lesions of grade 1] + 2x [No. of lesions of grade 2] + 3x [No. of lesions of grade 3]. The overall score is divided by a factor 10 (Main and Whitle, 1975). Mean score for each group was calculated and expressed as ulcer index. Percentage ulcer protection was calculated as follows (Suziki et al, 1976).

1- [Ulcer index with extract/ulcer index with Tween 20] x 100.

The procedure was repeated using petroleum ether (PE) extract [5% Tween 20 in Distilled water 5ml/kg, Cimetidine 100mg/kg and PE 200mg/kg].

2.5.3 INDOMETHACIN – INDUCED LESIONS:

The adult Wistar rats were fasted of food for 24 hours prior to the experiment but allowed free access to water. The rats were randomized and divided into three groups of four animals each according to the treatment employed [Distilled water 5ml/kg, cimetidine 100mg/kg, and ME 200mg/kg body weight]. Indomethacin 100mg/kg was administered orally 1hour after drug treatment. After 4hours of Indomethacin administration, the animals were sacrificed by blow on the head and their stomach removed and opened along the greater curvature then rinsed carefully under a running tap and pinned on a cork board and examined with a hand lens (x10). The number and severity of lesions were scored as below:

1..... less than or equal 1mm.

2..... 1-2mm.

3.....>2mm.

The ulcerative index (U.I) is calculated by using the formula, U.I = 1x [no. of lesions of grade 1] + 2x [no. of lesions of grade 2] + 3x [no. of lesions of grade 3]. The overall score is divided by a factor 10 (Main and Whitle, 1975). Mean score for each group was calculated and expressed as ulcer index. Percentage ulcer protection was calculated as follows (Suziki et al, 1976).

1- [Ulcer index with extract/ulcer index with Distilled water] x 100.

The procedure was repeated using petroleum ether (PE) extract [5% Tween 20 in Distilled water 5ml/kg, Cimetidine 100mg/kg and PE 200mg/kg].

2.5.4 STUDIES ON ISOLATED GUINEA PIG ILEUM

Adult guinea pigs were killed by cervical dislocation and bled. The abdomen was opened. Terminal portions of the ileum [2-3 cm] were used after discarding the portion nearest to the ileocaecal junction. The tissue was mounted in 20.0 ml organ bath containing Tyrode solution of the following composition per litre, NaCl 8 g, KCl 0.2 g, $CaCl_2$ 0.2 g, $NaHCO_3$ 1.0 g, $MgCl_2$ 0.05 g, NaH_2PO_4 1.0 g, and glucose 1.0 g. The solution was maintained at 37±1°C aerated with air. The resting tension on the tissue was 0.5 g. Sixty minutes equilibration period was allowed during which the physiological [Tyrode] solution was changed at every 15 minutes. At the end of the equilibration period, effects of graded concentrations of methanol and petroleum ether extracts and histamine and acetylcholine were determined with a kymograph. The contact time for each drug concentration was 1 minute, after which the tissue was washed three times to maintain 3 minutes cycles for the recovery of tissue. A resting period of 15 seconds was

allowed between drug additions. Inhibitory effect of the extracts on histamine and acetylcholine-induced contraction was also investigated.

2.5.5 STUDIES ON ISOLATED RABBIT JEJUNUM

The adult rabbits were killed by a blow on the head and bled. The abdomen was opened. The segments of the jejunum [2-3 cm] long were removed and dissected free of adhering mesentery. The lumen was flushed with Tyrode solution. The tissue was mounted in a 20 ml organ bath containing Tyrode solution at 37°C ± 1°C and aerated with air. A resting tension of 0.5 g was applied. The responses were recorded isometrically on an Ugo Basile Unirecord [7050] through Isometric transducer [7004] after an hour equilibration period during which the physiological solution was changed every 15 minutes. The effects of graded concentrations of acetylcholine and the crude extracts were evaluated. The contact time for each concentration of drug was 60 seconds, which was followed by washing three times. The tissue was allowed a resting period of 15 seconds between drug additions. Inhibitory effect of the extract on acetylcholine-induced contraction was investigated.

2.6 STATISTICAL ANALYSIS

Results were expressed, as mean ± SEM. Significance was determined using one-way ANOVA and student's t-test. Results were regarded as significant at $P \leq 0.05$.

CHAPTER THREE

3.0 RESULTS

3.1 PHYTOCHEMICAL TESTS.

The phytochemical evaluation of the methanol extract indicated the presence of saponins, reducing sugar, tannins, carbohydrates, flavonoids and glycosides with traces of alkaloids, steroids, proteins and terpenoids. The petroleum ether extract showed the presence of steroids, terpenoids, fats and oils with traces of tannins and flavonoids.

3.2 ACUTE TOXICITY STUDIES.

No lethality was observed in the mice upon oral administration of doses up to 5000 mg/kg. Furthermore, the extracts did not produce any obvious behavioral changes and no major clinical signs of toxicity (e.g. convulsion, respiratory distress) were observed in the animal during the 48 hours observation period.

3.3 DETERMINATION OF THE EFFECTIVE DOSE OF THE EXTRACTS.

The result obtained showed that both extracts are effective in inhibiting gastric lesions at doses from 200 mg/kg when compared with the inhibition of gastric ulcers with distilled water. Also, the results showed that the gastric ulcer inhibitions by both extracts at doses 200 mg/kg, 400 mg/kg and 800 mg/kg are virtually of equal effectiveness.

The result of the evaluation of the effective dose of the extracts is shown in the Table 1 and figure 2.1.

Table 1. Determination of the effective dose of the extracts

Absolute ethanol model/Treatment	Ulcer indices		
	200mg/kg	400mg/kg	800mg/kg
Methanol Extract	0.13+/- 0.10 [a]	0.08+/- 0.15 [a]	0.03+/- 0.05 [a]
Petroleum ether Extract	1.23+/- 0.89 [a]	0.33+/- 0.38 [a]	0.23+/- 0.29 [a]
Distilled Water (5 ml/kg)	7.43+/- 1.31 [b]	7.43+/- 1.31 [b]	7.43+/- 1.31 [b]

Mean data in the same column carrying different superscript differ significantly from each other ($P<0.05$).

3.4 Effect of the methanol extract on indomethacin and absolute ethanol-induced gastric lesions.

The results showed that the methanol extract produced significant ($P<0.05$) inhibition of gastric mucosal lesions compared with distilled water in both models. The result further indicated that the extract and cimetidine produced similar inhibitory effect on absolute ethanol and indomethacin-induced gastric lesions. Methanolic extract produced 88.51% inhibition while cimetidine produced 83.92% inhibition for indomethacin model. The extract also produced 85.41% and cimetidine produced 80.93% inhibition for absolute ethanol model.

The effect of the methanolic extract on indomethacin-induced and absolute ethanol-induced gastric lesions is shown in Table 2 and figure 2.2.

Table 2. Effect of the methanol extract on indomethacin-induced and absolute ethanol-induced gastric lesions.

Treatment	Models/Ulcer indices	
	Indomethacin	Absolute ethanol
Water	6.53+/- 0.47^{B}	6.03+/- 1.16^{B}
Cimetidine	1.05+/- 0.42^{A}	1.15+/- 0.91^{A}
Methanol Extract (200 mg/kg	0.75+/- 0.97^{A}	0.88+/- 0.62^{A}

Mean data in the same column carrying the same superscript do not differ significantly from each other ($P>0.05$).

3.5 Effect of petroleum ether extract on indomethacin and absolute ethanol-induced gastric mucosal lesions.

The results showed that the petroleum ether extract produced significant ($P<0.05$) inhibition of gastric mucosal lesions compared with distilled water in both models. The result further indicated that the extract and cimetidine produced similar inhibitory effect on absolute ethanol and indomethacin-induced gastric lesions. Petroleum ether extract produced 84.26% inhibition while cimetidine produced 87.81% inhibition for indomethacin model. It also produced 71.74% while cimetidine produced 88.56% inhibition for absolute ethanol model.

The effect of the petroleum ether extract on indomethacin-induced and absolute ethanol-induced gastric lesions is shown in Table 3 and figure 2.3.

Table 3. Effect of petroleum ether extract on indomethacin and absolute ethanol-induced gastric mucosal lesions.

Treatment	Models/Ulcer indices	
	Indomethacin	Absolute ethanol
Water	8.45+/- 0.26[a]	7.43+/- 1.31[a]
Cimetidine	1.03+/- 0.89[c]	0.85+/- 0.34[c]
Petroleum ether Extract (200 mg/kg)	1.33+/- 0.87[c]	2.10+/- 0.48[c]

Mean data in the same column carrying different superscript differ significantly from each other ($P<0.05$).

3.6 Studies on isolated smooth muscles preparations.

The extracts showed no effect on the guinea pig ileum. Histamine and Acetylcholine produced a concentration dependent contraction of the guinea pig ileum. However, both extracts inhibited the contractile effects of histamine and acetylcholine in a concentration-dependent pattern (figures 2.4a, 2.4b, 2.5a, 2.5b, 2.6a, 2.6b, 2.7a, 2.7b).

On rabbit jejunum, acetylcholine produced a concentration dependent contraction (figure 2.8a and figure 2.8b) while both extracts caused a concentration dependent relaxation of the spontaneous contraction (figure 2.9a and figure 2.9b). The extracts also attenuated the acetylcholine-mediated contraction of the rabbit jejunum (figure 2.9a and figure 2.9b).

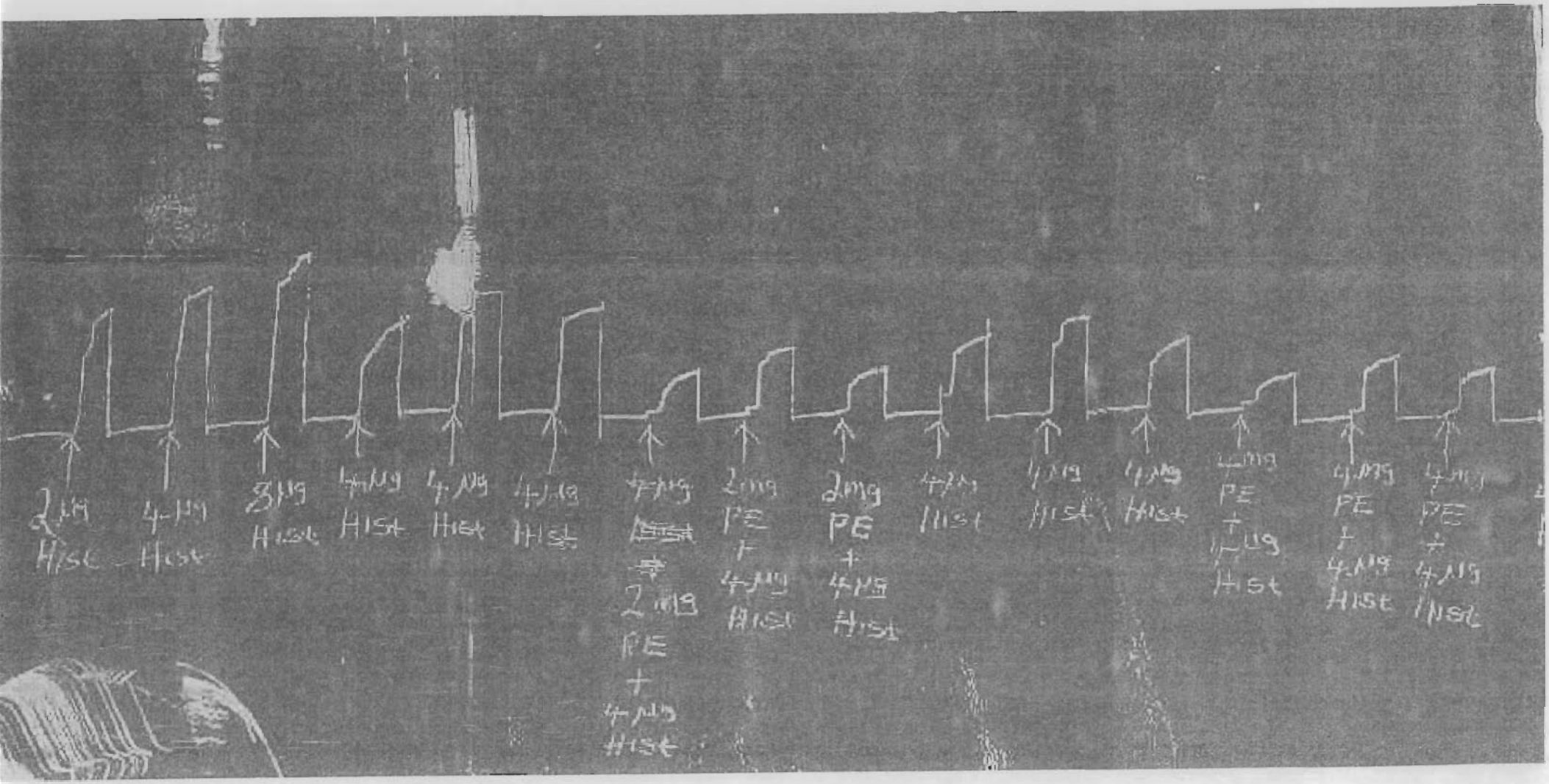


Fig 2.4a Effect of the Petroleum Ether extract on Histamine-induced contraction of the Guinea Pig ileum.

EFFECT OF THE PET/ETHER EXTRACT (PE) ON THE CONTRACTION EVOKED BY HISTAMINE ON GUINEA PIG ILEUM

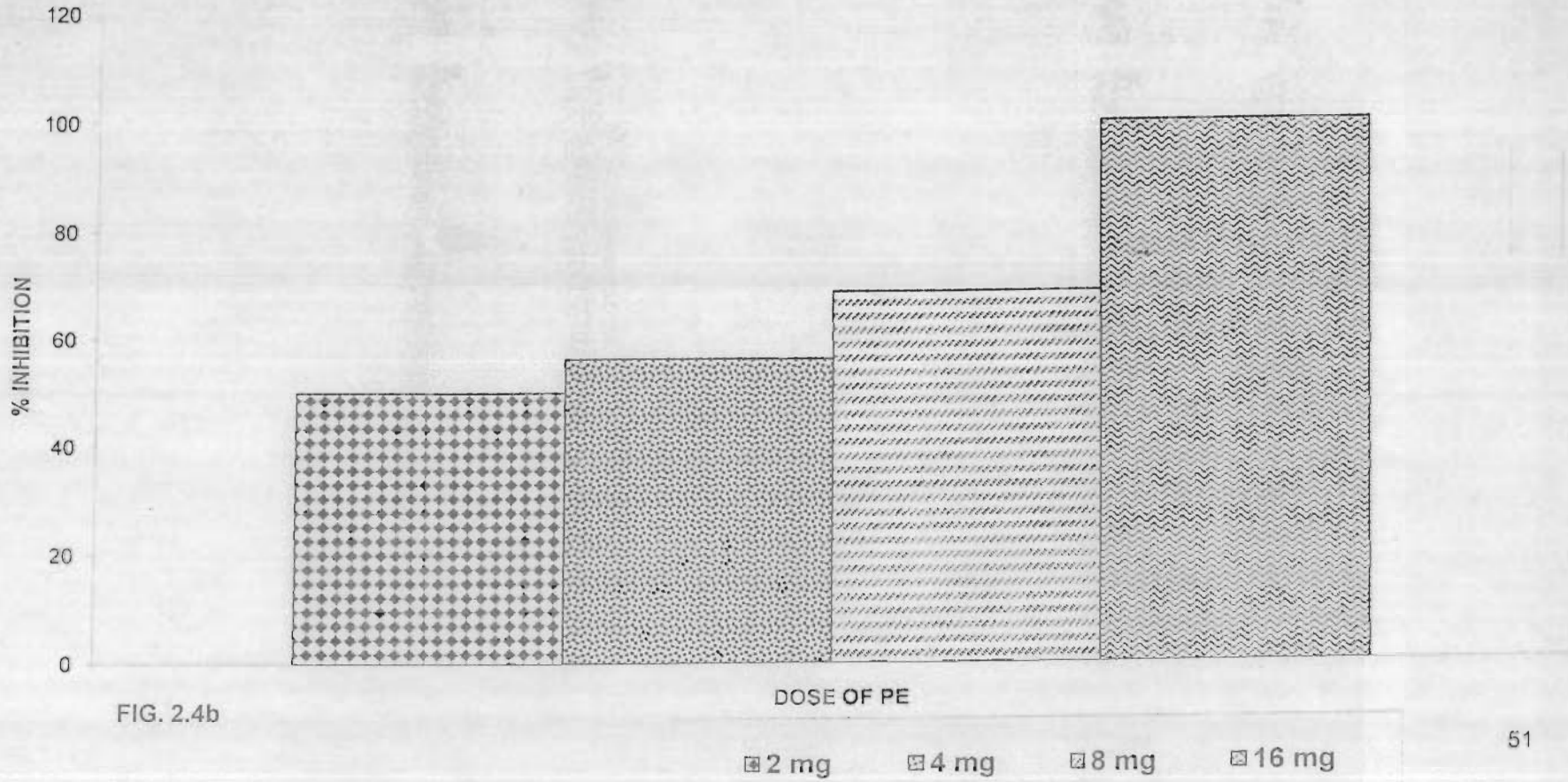


FIG. 2.4b

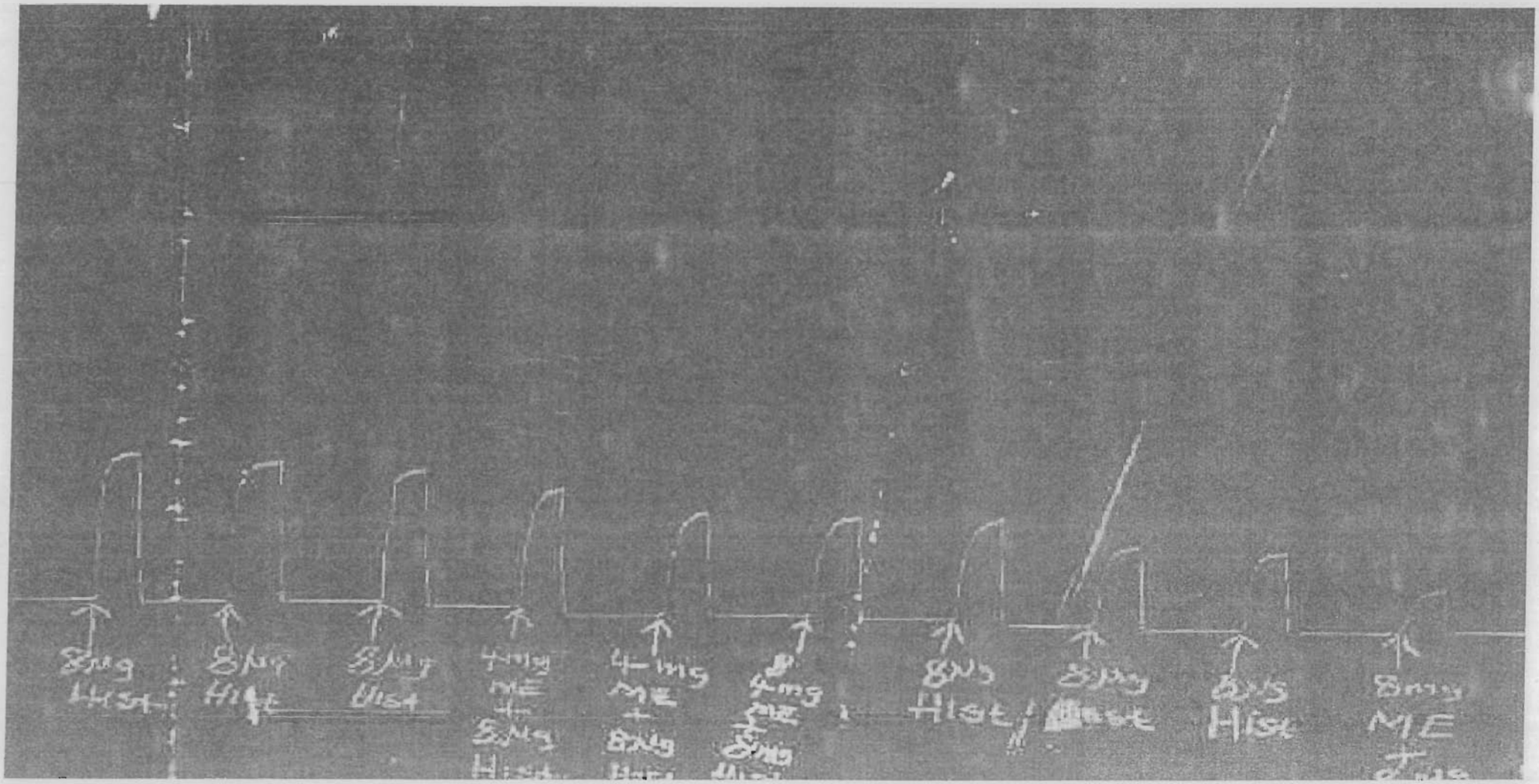


Fig 2.5a Effect of the Methanol extract on Histamine-induced contraction of the Guinea Pig ileum.

EFFECT OF THE METHANOL EXTRACT (ME) ON THE CONTRACTION EVOKED BY HISTAMINE ON GUINEA PIG ILEUM

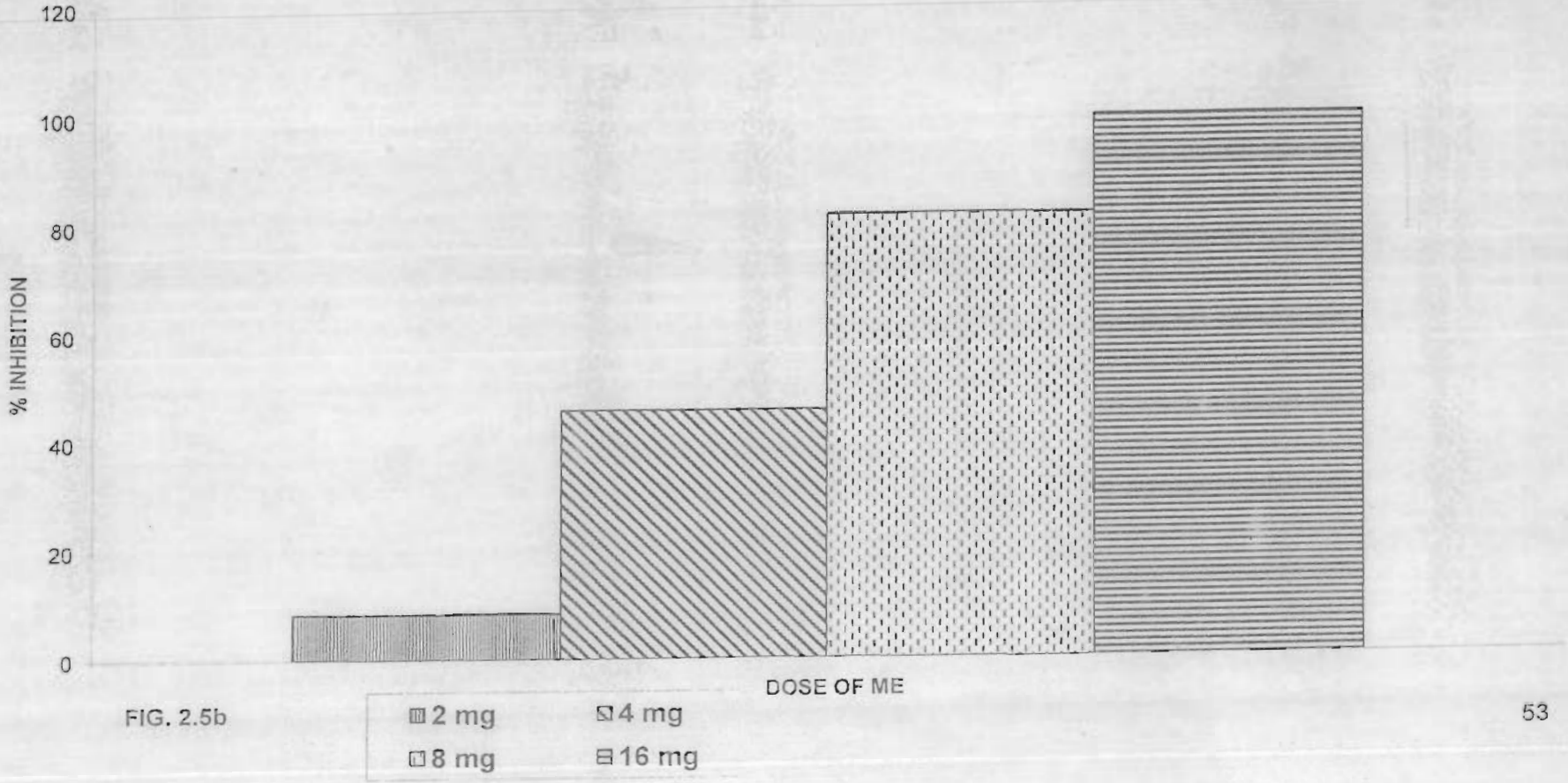
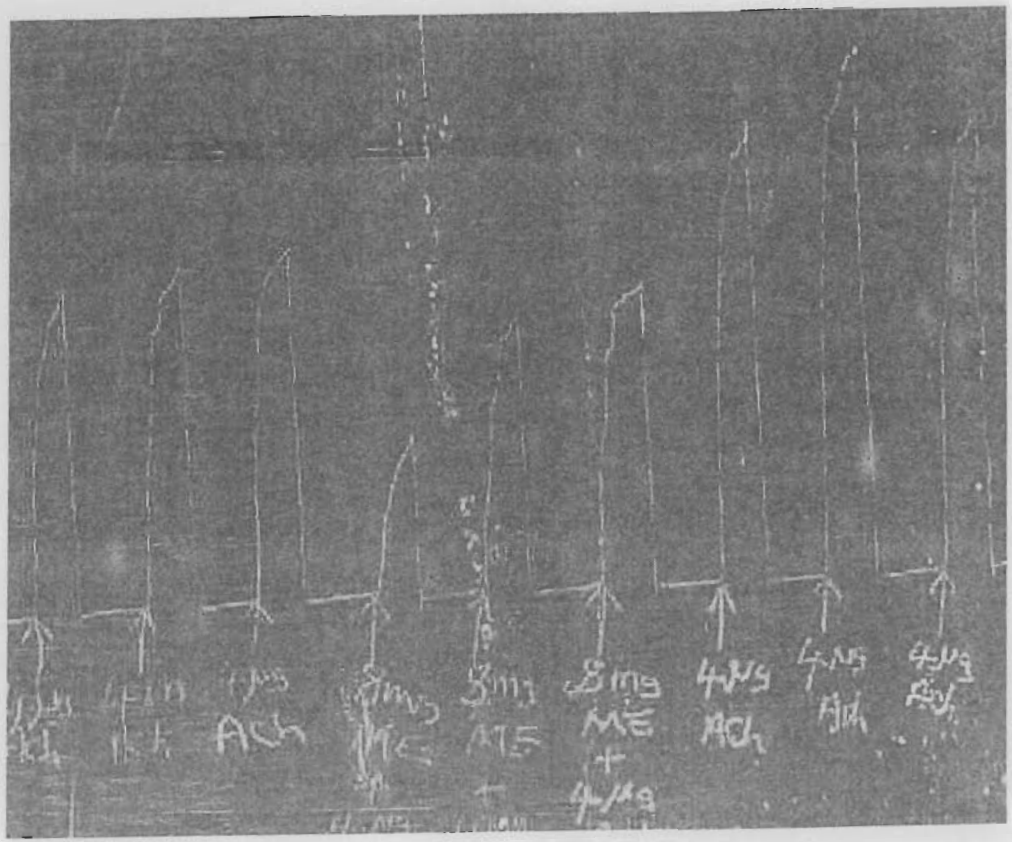


FIG. 2.5b



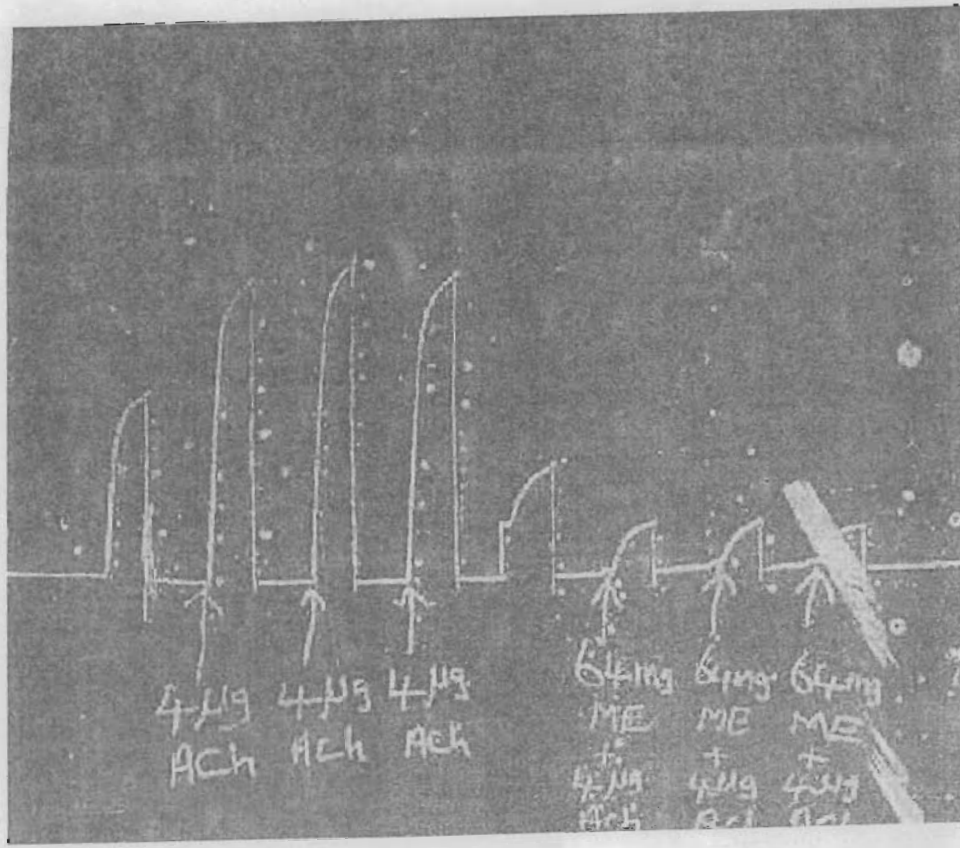


Fig 2.6a Effect of the Methanol extract on Ach-induced contraction of the Guinea Pig ileum.

EFFECT OF THE METHANOL EXTRACT (ME) ON CONTRACTION EVOKED BY ACETYLCHOLINE (Ach)

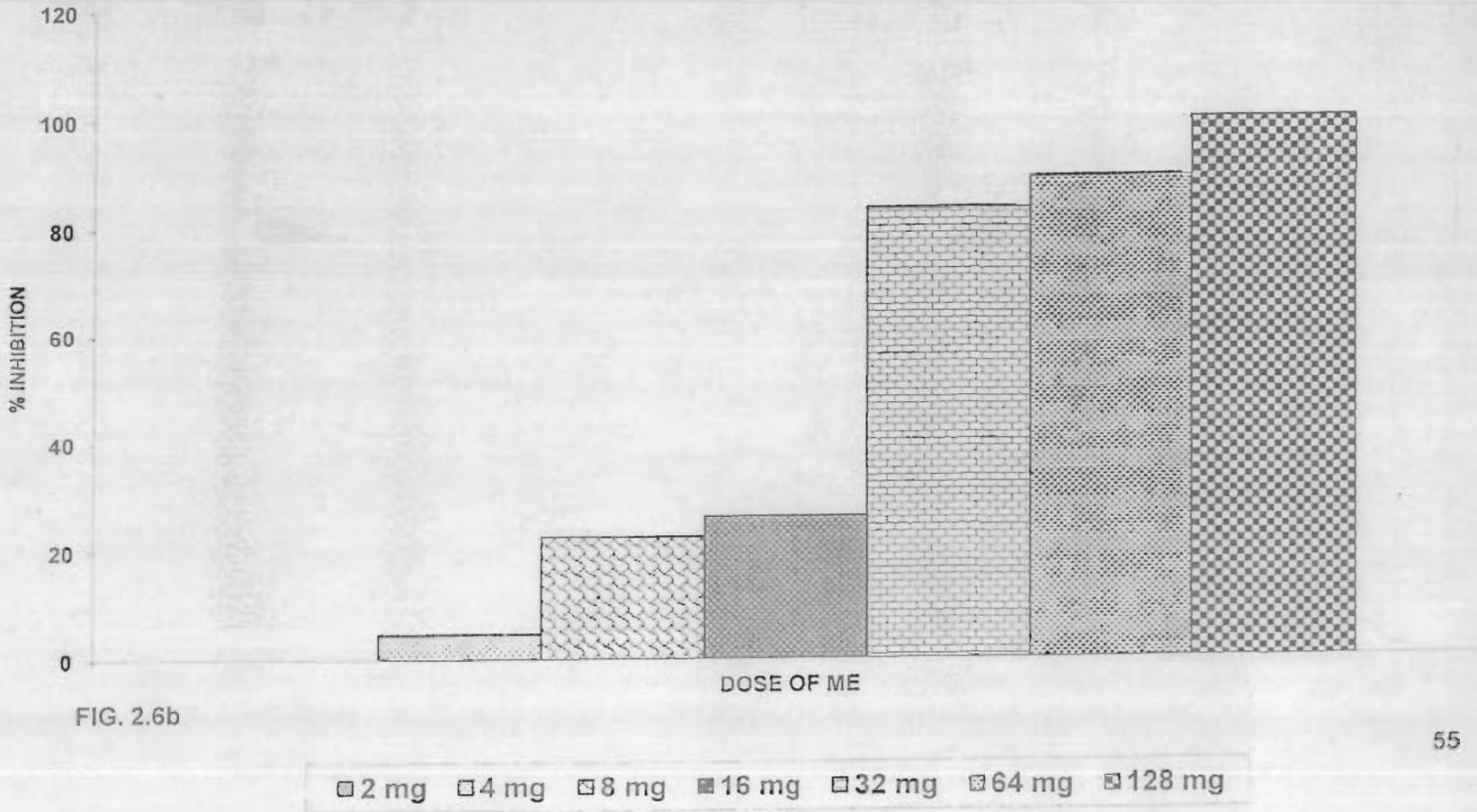


FIG. 2.6b

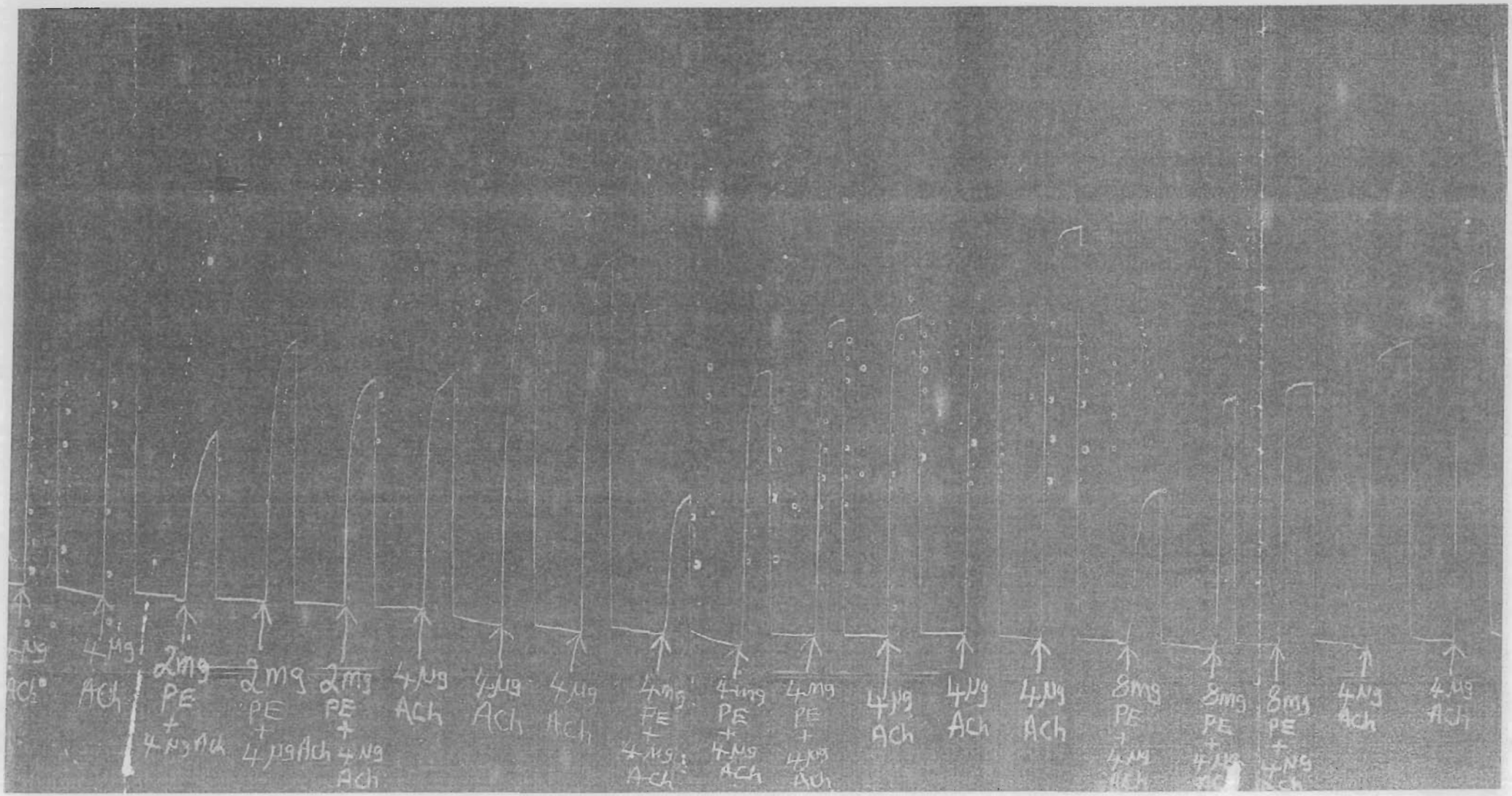


Fig 2.7a Effect of the Petroleum Ether extract on Ach-induced contraction of the Guinea Pig ileum.

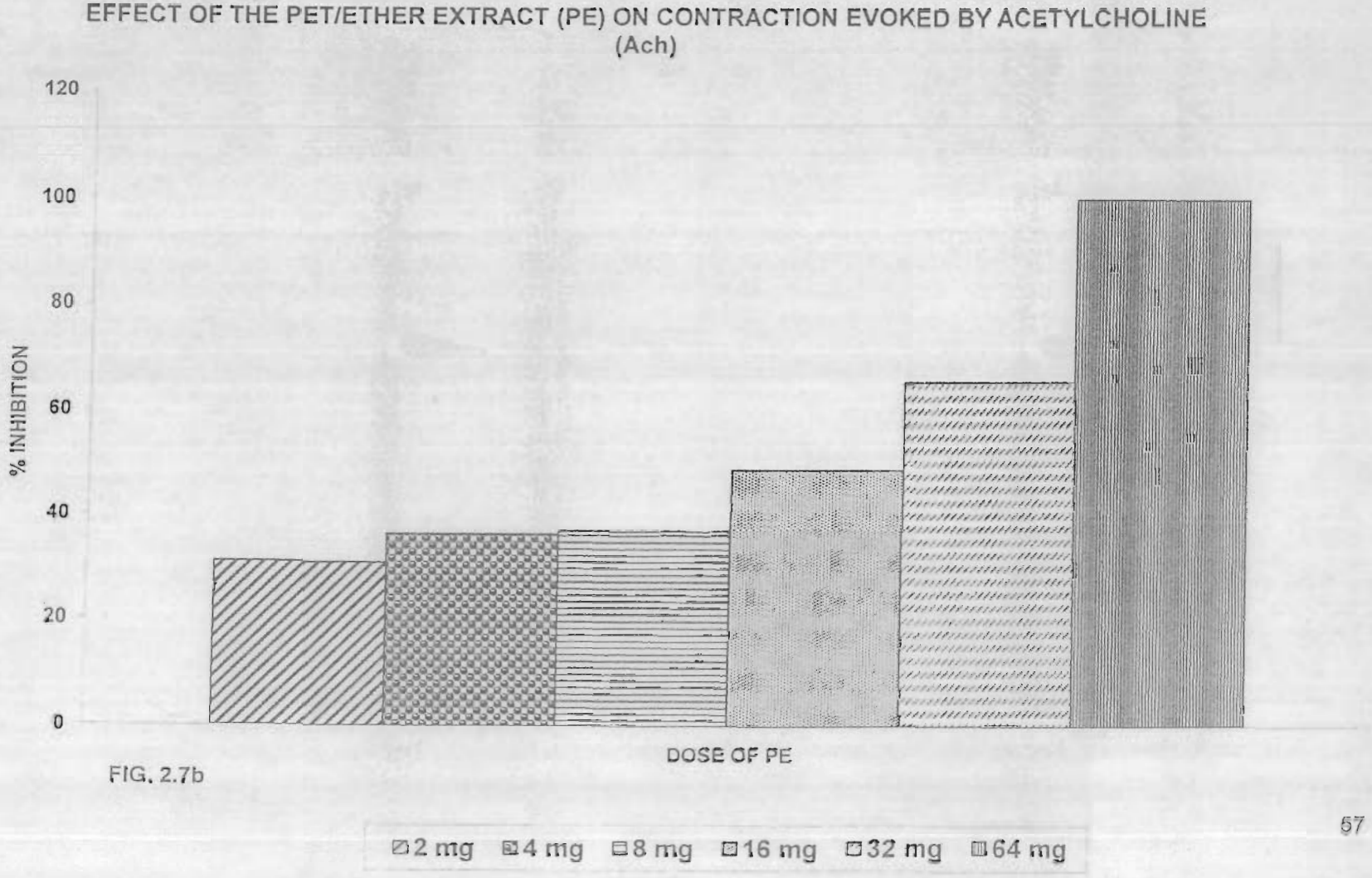

EFFECT OF THE PET/ETHER EXTRACT (PE) ON CONTRACTION EVOKED BY ACETYLCHOLINE (Ach)
120
100
80
60
40
20
0
% INHIBITION
DOSE OF PE
2 mg
4 mg
8 mg
16 mg
32 mg
64 mg

FIG. 2.7b

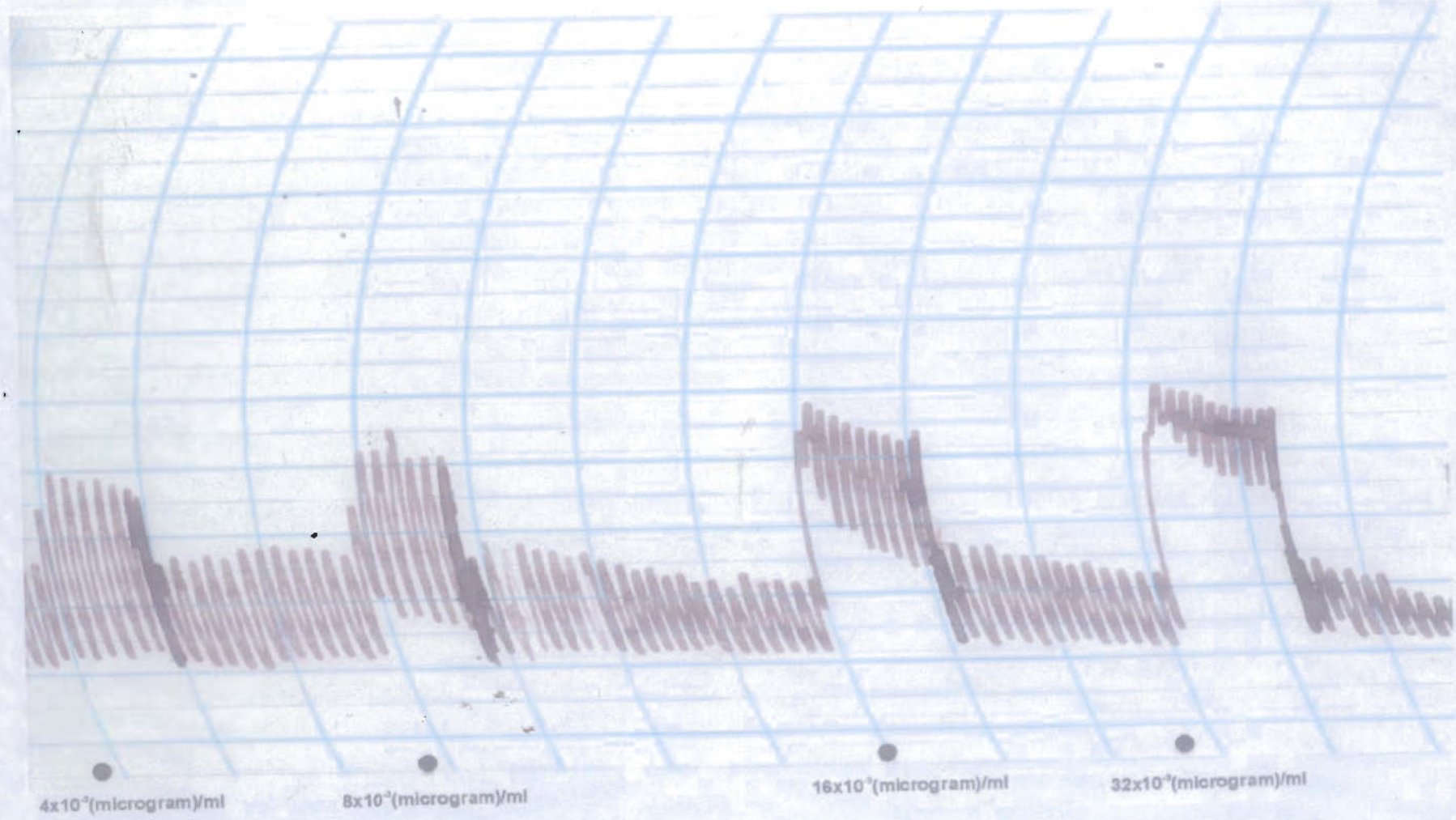


Fig: 2.8a Effect on Ach alone on the rhythmic Spontaneous Contraction of rabbit jejunum

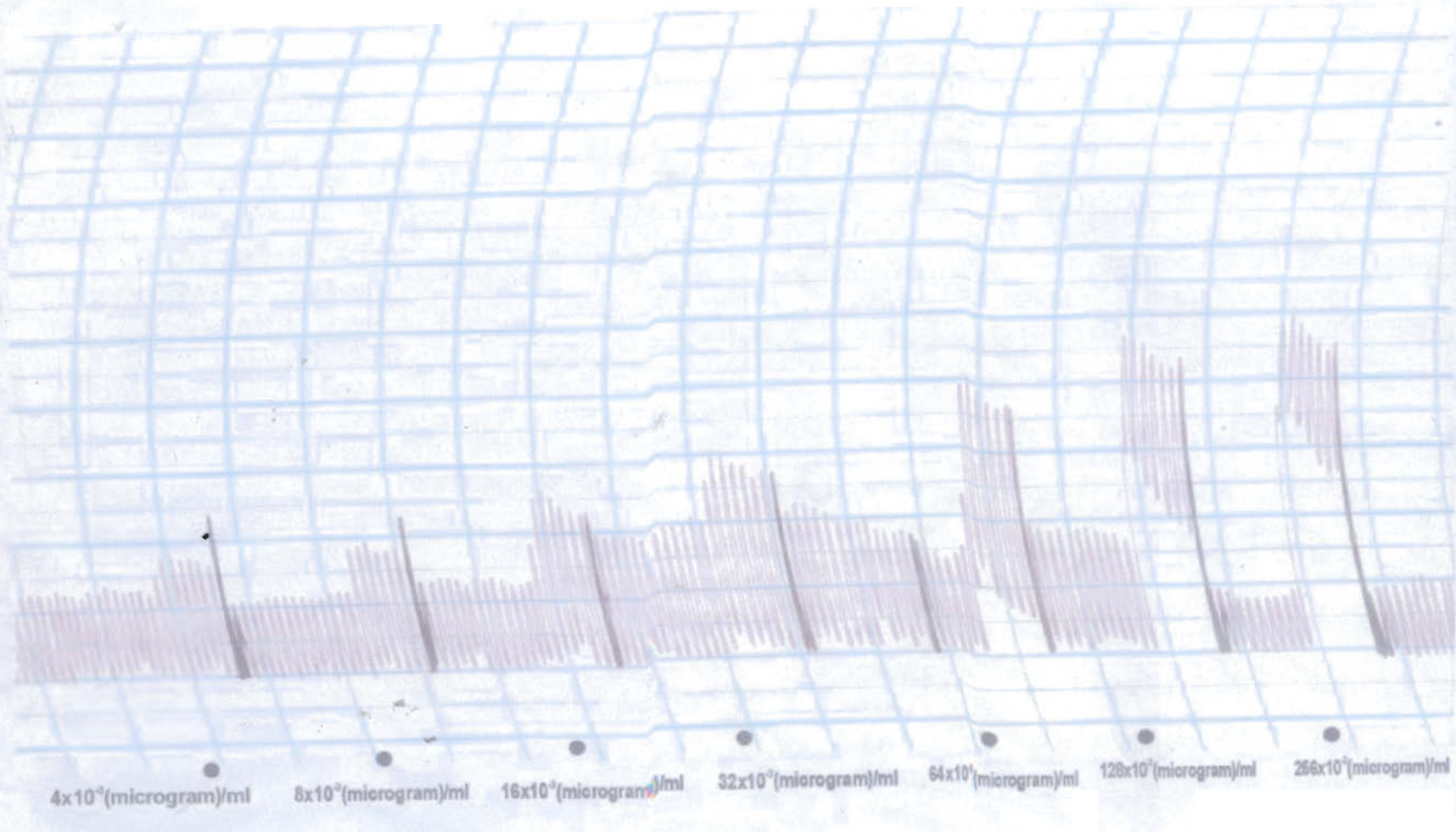


Fig: 2.8b Effect of the Ach alone on the rhythmic spontaneous contraction of the rabbit jejunum.

*Microgram/ml = dose of Ach

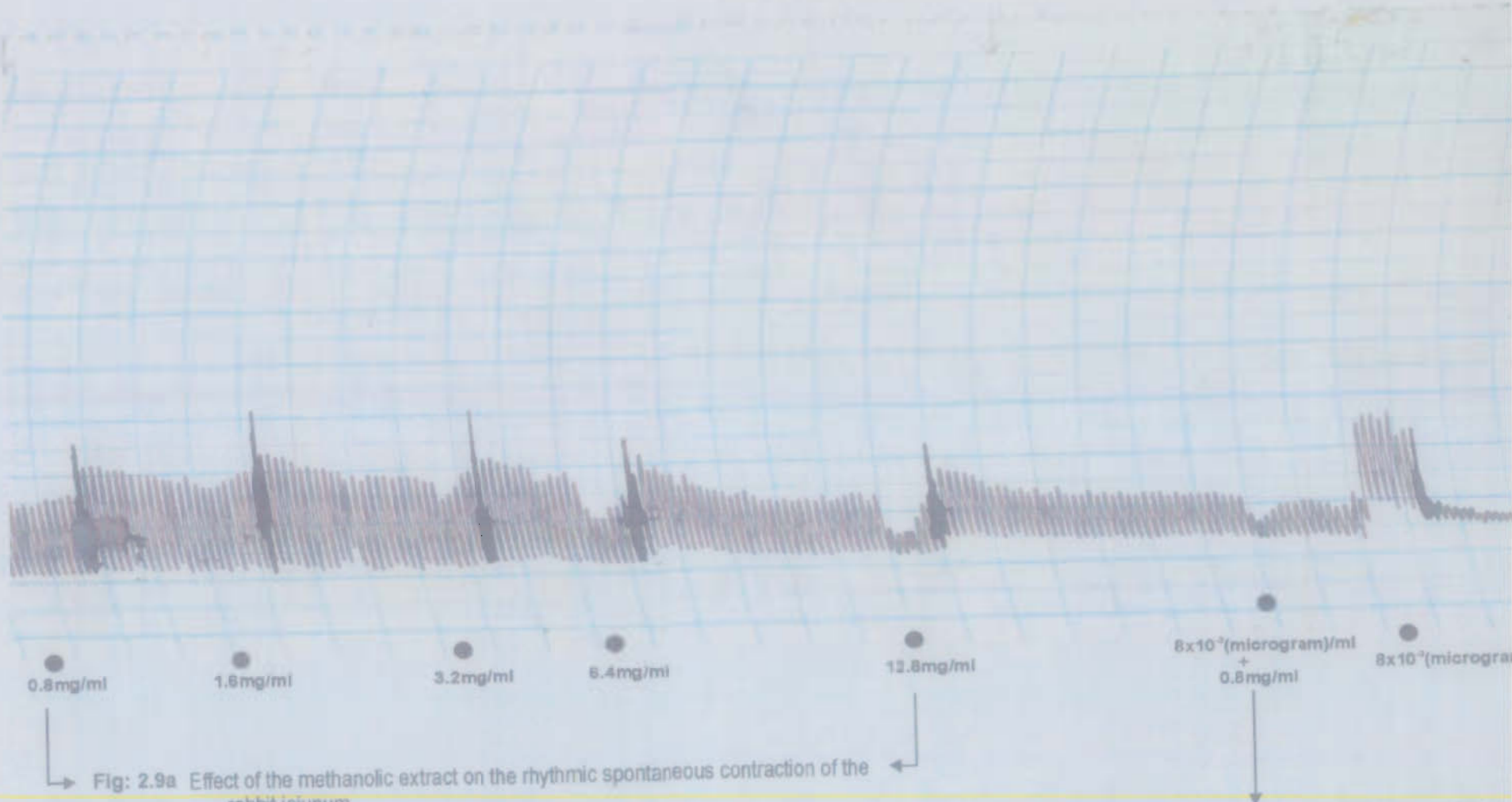


Fig: 2.9a Effect of the methanolic extract on the rhythmic spontaneous contraction of the rabbit jejunum.

* mg/ml = dose of the methanolic extract
microgram/ml = dose of Ach

Effect of the methanolic extract on the Ach pre-contracte
rabbit jejunum

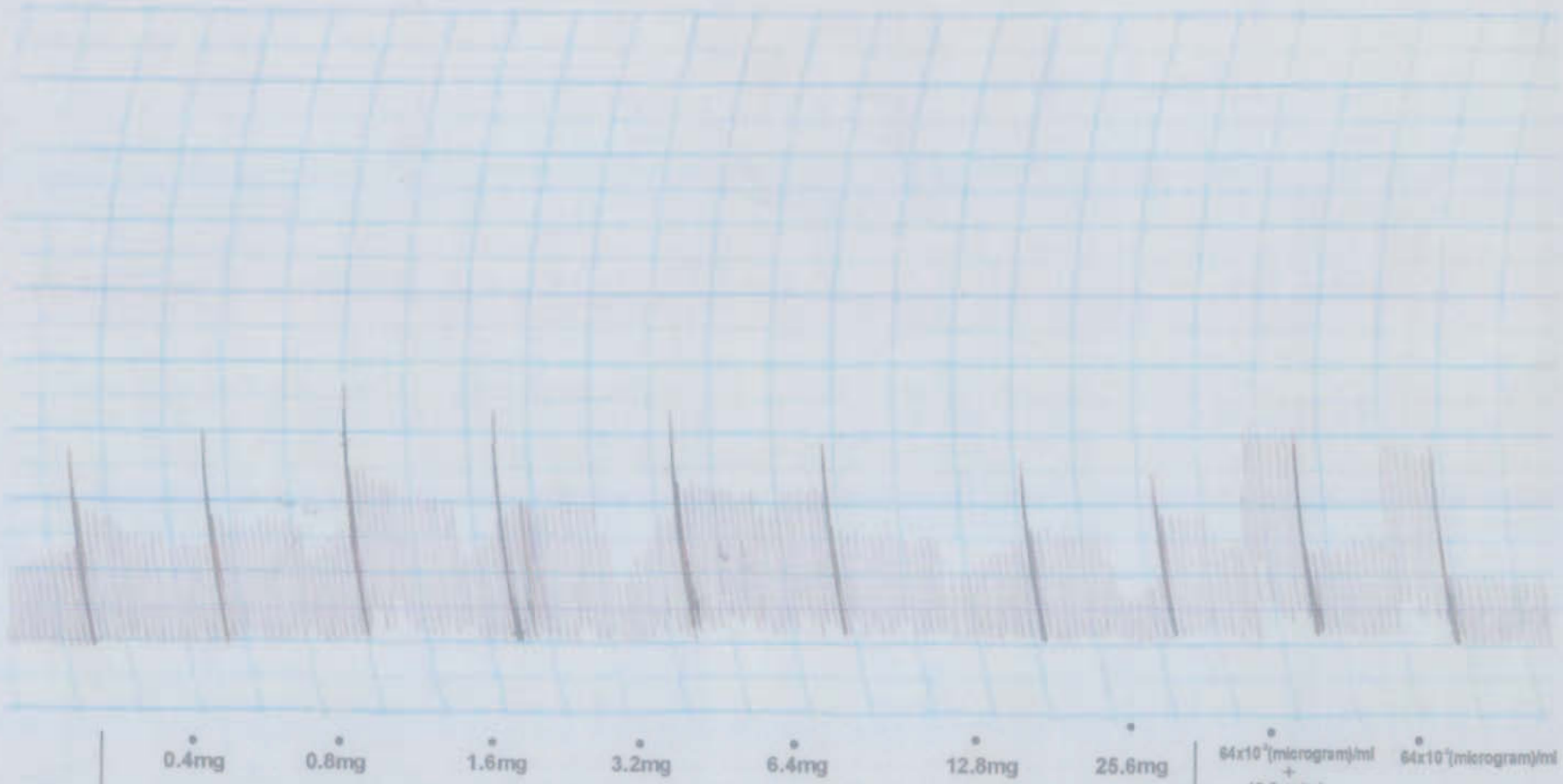


Fig: 2.9b Effect of the Petroleum Ether extract on the rhythmic spontaneous contraction of the rabbit jejunum.

* mg/ml = dose of the Petroleum Ether extract
microgram/ml = dose of Ach

Effect of the Petroleum Ether extract on the Ach pre-contracted rabbit jejunum

CHAPTER FOUR

4.0 DISCUSSION.

The study has provided data that both methanol and petroleum ether extracts of *Bridelia ferruginea* possess biologically active constituents with anti-ulcerogenic properties. Anti-ulcerogenic activity of substances can be evaluated using some common models such as absolute ethanol-induced, indomethacin-induced, reserpine-induced and hypothermic restraint stress-induced ulcers in rats (Bighetti et al, 2005).

There are different mechanisms of formation of gastric lesions in each of these models (Parmar and Ghosh, 1981). Ethanol produces gastric mucosal lesions by direct toxic effect on the mucosa, by depleting the mucus and inhibition of the secretion of bicarbonate (Marhuenda et al, 1993 and Koo et al, 1986). Furthermore, ethanol increases vascular permeability (Szabo et al, 1986), leukotrienes (Goel, 2002), the release of histamine, influx of calcium ions and generation of free radicals (Galvin and Szabo, 1992). Moreover, ethanol reduces the levels of endogenous antioxidant, glutathione, and cytoprotective prostaglandin (Szabo et al, 1981).

Studies focusing on the pathogenesis of ethanol-induced gastric mucosal injury suggest that an initial event is disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting (Ajaikumar et al, 2005).

Non-steriodal antiinflamatory drugs (NSAID) cause mucosal damage through mechanisms, which involve the inhibition of the biosynthesis of prostaglandin as well as the enhancement of the production of leukotrienes. Prostaglandins inhibit acid secretion and stimulate the secretion of mucus, bicarbonate and blood flow (Ibu et al, 1994). Several studies have indicated that gastroduodenal protection by prostaglandins involves increase in mucosal resistance and decrease in aggressive factors mainly acid and pepsin

(Aly, 1987). Inhibition of prostaglandin synthesis by indomethacin coincides with the earlier stages of damage to cell membranes of mucosal, parietal and endothelial cells (Rainsford, 1984).

The result obtained from the study showed that the extracts protected the rats against ethanol and indomethacin-induced gastric lesions. The protective effects of the extracts may be due to cytoprotective activity. The models used for the evaluation are used to screen drugs for cytoprotection (Robert, 1979, Morimoto et al, 1991). This cytoprotective mechanism may occur as result of the ability of some compounds to induce prostaglandin production, which is fundamental for mucus protection as prostaglandins stimulate mucus and bicarbonate synthesis (Robert et al, 1983).

High percentage inhibition of the ulceration lesion index was observed reinforcing the hypothesis that anti-ulcerogenic substances present in the extract act through a specific systemic mechanisms.

The extracts showed dose-dependent inhibition of the histamine-induced contraction of isolated guinea pig ileum. The H_2 receptors of the parietal cells when stimulated by histamine increase CAMP which will activate the protein kinase A, the enzyme that triggers sequential events that lead to acid secretion (Garcia et al, 1978; Solcia et al, 1993). This implies that the extracts possibly act by blocking histamine H_2 receptors or influence intracellular histamine activity.

Also, they inhibited the acetylcholine-evoked contraction of guinea pig ileum. The extracts also caused dose-dependent inhibition of the intrinsic rhythmic contraction of rabbit jejunum. They further inhibited the potentiation of the intrinsic rhythmic contraction of rabbit jejunum by acetylcholine in a dose-dependent pattern.

The vagus nerve stimulates stomach acid secretion via interaction of a chemical mediator, acetylcholine, with muscarinic receptors, which are located at parietal cells and histamine secretory cells (Barocelli et al, 1997; Schubert, 2000). Therefore the blockade of the acetylcholine activity by the extracts suggested an anticholinergic mechanism or an interruption of events that are linked to acid secretion.

The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins and saponins. The anti-ulcer activity of both extracts may be attributed to the presence of these constituents. Alkaloids affect the integrity of the mucus membrane (Oliver, 1960). Some extracts may contain alkaloids without exhibiting anti-ulcer activity due to alkaloids' multivariate property (Nwafor and Akah, 2003). Also, the presence of flavonoids may account for the anti-ulcerogenic property. This is because flavonoids possess antibacterial, spasmolytic as well as the ability to inhibit acid secretion (Nwafor and Akah, 2003 ; Carlo et al, 1994; Evans, 1989). Furthermore, flavonoids influence the metabolism of arachidonic acid, have vasoprotective activity and interfere with the formation of histamine in the gastric mucosa (Parmar and Ghoshi, 1976; Akah et al, 1998).

The presence of tannins, which are astrigent, vasoconstrictor, and precipitator of proteins further enhance the anti-ulcer activity of the extracts. Tannins may precipitate micro proteins at the site of the ulcer, which forms an impervious protective pellicle over the lining to render it less permeable to toxic substances and more resistant to attack of proteolytic enzymes (Nwafor et al, 2000).

Triterpene-saponins possess anti-ulcer activity through the formation of mucus and inhibitory action on $PGF_{2\text{-alpha}}$ (Lewis and Hanson, 1991; Aguwa and Okunji, 1986).

Though the methanol extract has higher percentage protection against ulcer than the petroleum ether extract, this is not statistically different. This may be attributed to the higher composition of the extract with saponins, tannins and flavonoids.

It can be concluded from these results that *Bridelia ferruginea* exhibits pharmacological effects related to ulcer management and contains active ingredients with a therapeutic potential against gastric ulcers. *Bridelia ferruginea* may be of value in the development of new therapy for the treatment of peptic ulcer diseases.

EFFECTIVE DOSE OFTHE EXTRACTS USING ABSOLUTE ETHANOL MODEL

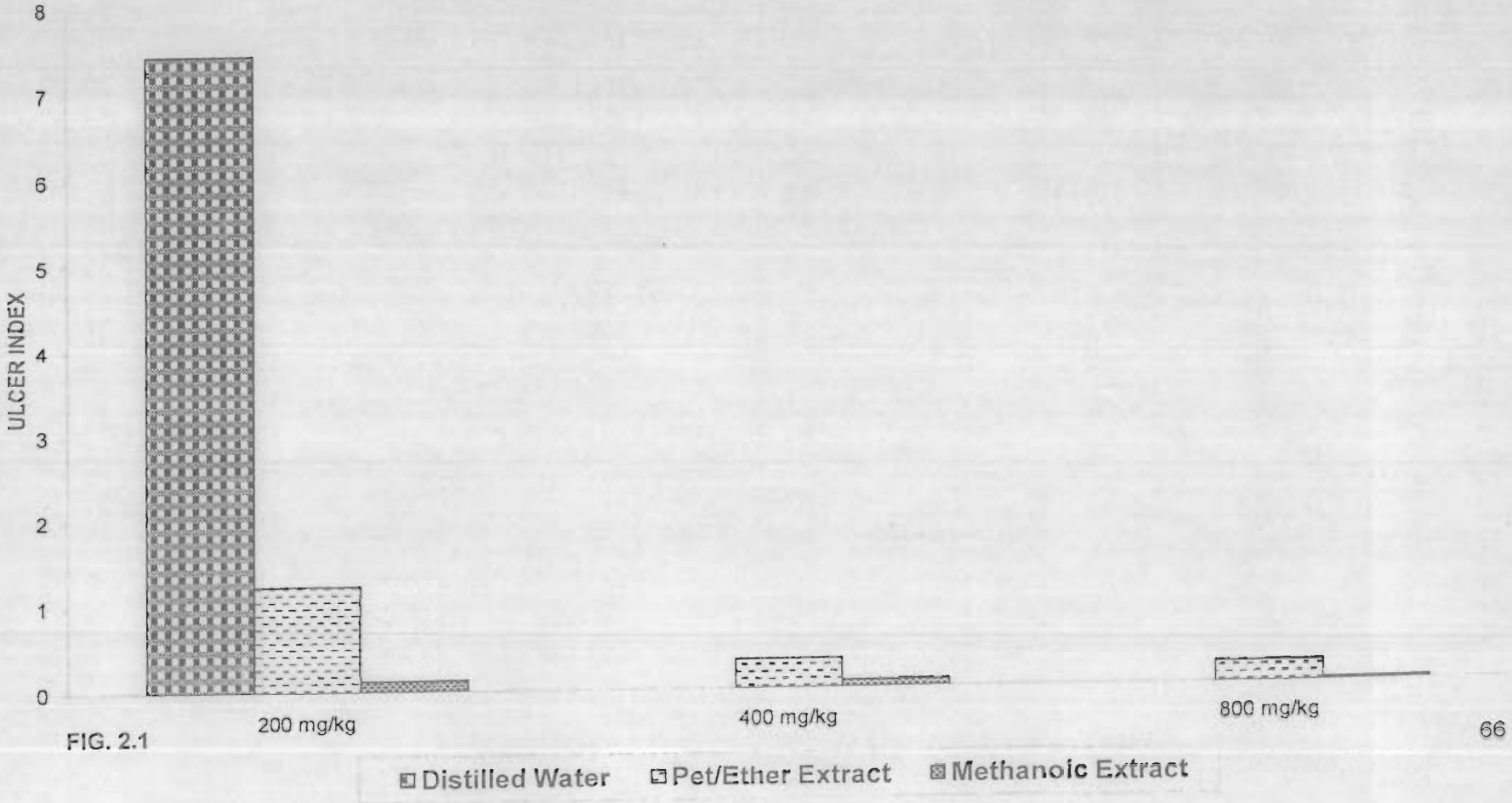


FIG. 2.1

THE EFFECT OF THE METHANOL EXTRACT (ME) ON ULCERS INDUCED BY INDOMETHACIN AND ABSOLUTE ETHANOL

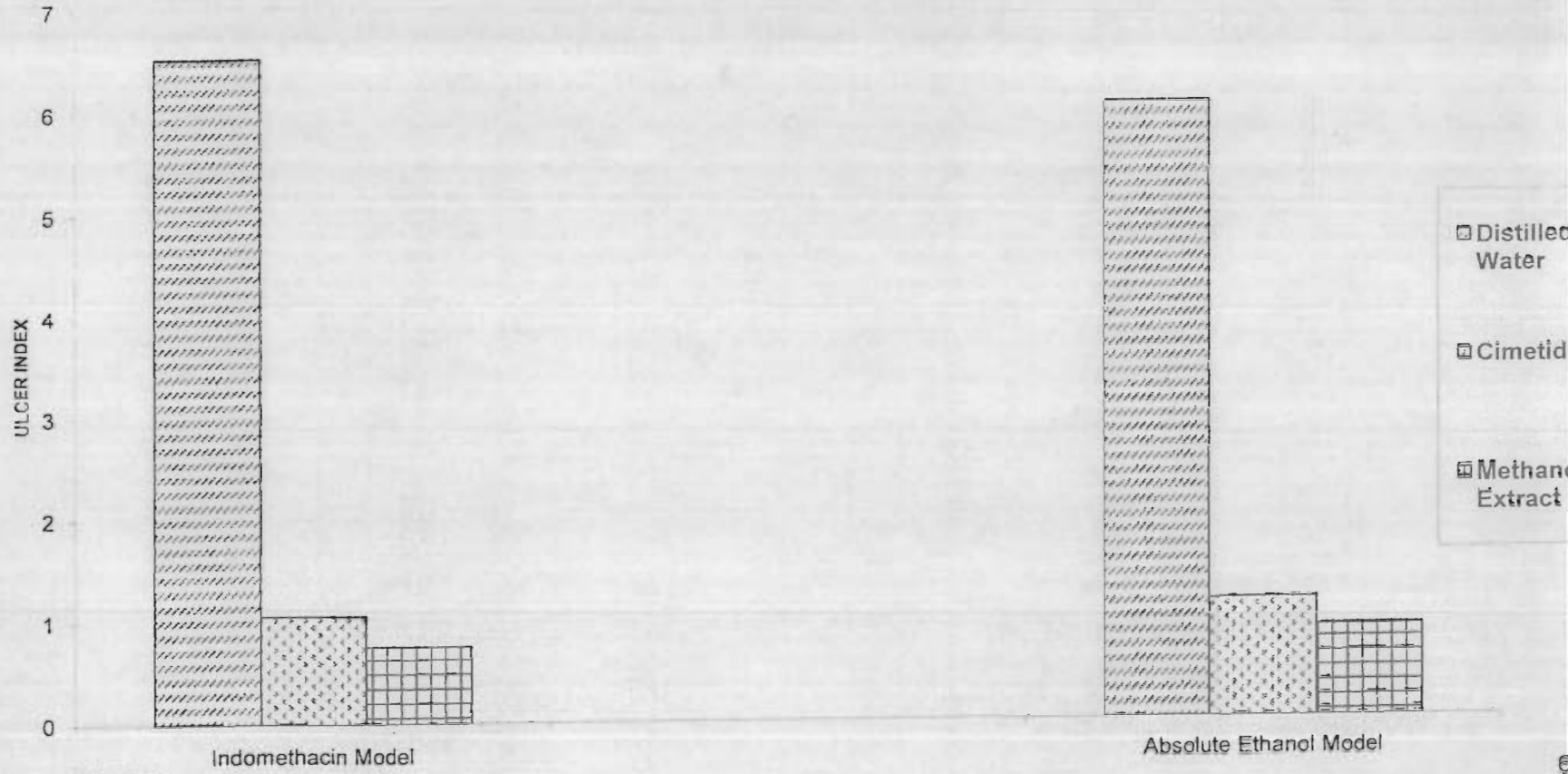


FIG. 2.2

APPENDIX III

THE EFFECT OF THE PET/ETHER EXTRACT (PE) ON ULCERS INDUCED BY INDOMETHACIN AND ABSOLUTE ETHANOL

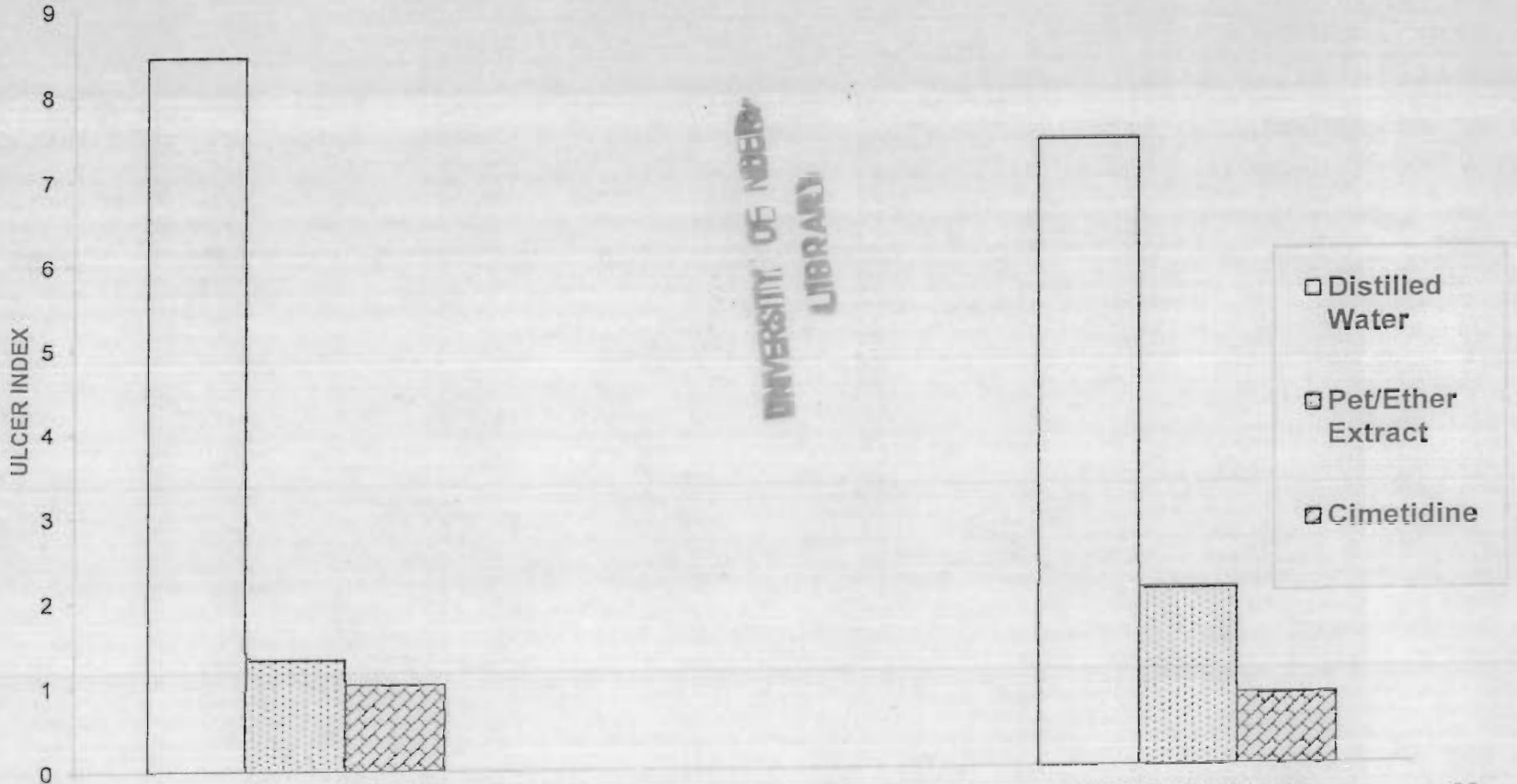


FIG. 2.3

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