MECHANISM OF ANTI-GASTRIC ULCER EFFECT OF BETULINIC ACID IN MALE ALBINO RATS

BY

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY, COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN, IN PARTIAL FULFILLMENT OF REQUIREMENT FOR THE MASTER OF PHILOSOPHY (PHYSIOLOGY) (MPhil.) DEGREE.

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Certification Page

I certify that this work was carried out by Mr Chinedu Onwuchekwa in the Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria under my supervision

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DEDICATION

This project is dedicated to

GOD ALMIGHTY

For His abundant grace and merciful kindness

to me.

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ACKNOWLEDGEMENTS

My appreciation goes first to God almighty for His grace towards me and sparing my life till this day.

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ABSTRACT

Betulinic acid (BA) is a lupane-type triterpene that has been identified and isolated from different plant species used in ethnomedicine worldwide. It is known to possess anti-ulcer, anti-tumor and anti-microbial properties. However, the mechanism underlying the anti-ulcer property of BA has not been well investigated. The effect of BA on indomethacin-induced peptic ulcer, gastric mucus secretion (GMS), gastric mucus cell count (GMCC), basal and histamine-induced gastric acid secretion (GAS), and malondialdehyde (MDA) concentration level were studied as means of elucidating the mechanism.

Thirty-two rats divided into four groups of eight rats each were used for each study. Group I (Control) was pretreated orally with dimethyl sulfoxide (DMSO) in normal saline for seven days. Groups II, III and IV were pretreated orally with BA (0.5 mg/kg, 1.5 mg/kg, and 3.0 mg/kg) dissolved in DMSO respectively for seven days. Gastric ulceration was induced using indomethacin and scored by ulcer scoring technique. Measurement of GMS was performed using spectrophotometric method, while GMCC was done by using calibrated microscopy. Continuous perfusion technique was used to assess GAS and its acidity determined by titration. Histological study of the stomach mucosa was also carried out. MDA levels were determined by measuring thiobarbituric acid reactive substances produced. Data

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were expressed as Mean ± SEM. Student's t-test and one way ANOVA were used to determine levels of significance at $p<0.05$.

There was a dose - dependent reduction in ulcer scores in the BA treated animals. This reduction was significant at doses of 1.5mg/kg (0.75 ± 0.10) and 3.0mg/kg (0.25 ± 0.09) compared to the control (7.0 ± 0.27). GMS (mg/g tissue x 10^{-2}) significantly increased in the 1.5 mg/kg (4.9 ± 0.22) and 3.0 mg/kg (5.2 ± 0.09) pretreated groups compared to the control (4.4 ± 0.20) group. There was also a dose-dependent significant increase in the GMCC/mm^2 of rats treated with 0.5 mg/kg (44.4 ± 0.84); 1.5 mg/kg (45.9 ± 0.79); 3.0 mg/kg (54.1± 0.71)] compared with the control group (41.4 ± 0.71). The histamine-induced GAS was significantly higher than the basal GAS. However, there was a significant decrease in GAS of BA treated groups compared with the control. Histological study showed significant hyperplasia of mucus cells in BA treated groups compared to the control group. There was a significant decrease in MDA (μmol/L x 10^{-6}) levels in groups II, III and IV (1.86± 0.076, 1.83 ± 0.069 and 1.10 ± 0.056) compared to the control (2.60 ± 0.110) group.

The results of the study suggest that betulinic acid produces anti-ulcer effect by increasing gastric mucus secretion, gastric mucus cell count and decreasing malonialdehyde concentration. It decreases gastric acid secretion via the blocking of H_2-receptors.

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Key words: Betulinic acid, gastric mucus secretion, malondialdehyde, gastric ulcer

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CHAPTER ONE

1.0 INTRODUCTION

Natural products have been used for combating human diseases for thousands of years, since they exhibit a wide range of biological properties that can be exploited for medical application (Newman *et al,* 2003).

. In fact, the majority of anti-infectious and anticancer agents are of natural origin. Natural products of plant origin are a major part of traditional medical system in developing countries and also in herbal remedies in the western countries. This has motivated research for plants with potential therapeutic activity. It is against the foregoing background of the socio-economic status of developing countries, the magnitude of their health problems and the few resources available that any advantage of natural product usage can be and should be viewed. Natural products are more accessible to most of the population in the third world and cheaper than those manufactured by modern health technology in most cases. It has a wider acceptability among the people in developing countries than synthetic drugs in that

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it blends readily into the system. As far as life is concerned the subject of priority is health. But despite efforts to maintain good health, man and animals alike still confront disease conditions which are due to exposure to physiopathological agents (Sofowora, 1982). Such agents include microorganisms, noxious substances, etc in the environment. Tobacco smoking leads to atherosclerosis and vascular spasms, causing vascular insufficiency and promoting the development of ulcers through ischemia. Nicotine contained in cigarettes can increase parasympathetic nerve activity to the gastrointestinal tract by acting on the nicotinic receptors at synapses - increased stimulation to the enterochromaffin-like cells and gastric (G) cells increases the amount of histamine and gastrin secreted and therefore increases the acidity of the gastric juice. Similarly, glucocorticoids lead to atrophy of all epithelial tissues. However, these factors, along with diet or spices, blood type, and other factors suspected to cause ulcers until late in the 20th century, are actually of relatively minor importance in the development of peptic ulcers (National Digestive Diseases Information Clearing House, 1999). A major causative factor (60% of gastric and up to 90% of duodenal ulcers) is chronic inflammation due to Helicobacter pylori that colonizes the antral mucosa. The immune system is unable to clear the infection, despite the appearance of antibodies. Thus, the bacterium can cause a chronic active gastritis (type B gastritis), resulting in a defect in the regulation of gastrin production by that part of the stomach, and gastrin secretion

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can either be decreased (most cases) resulting in hypo- or achlorhydria. Gastrin stimulates the production of gastric acid by parietal cells and, in H. pylori colonization, responses that increase gastrin and the increase in acid can contribute to the erosion of the mucosa and therefore ulcer formation.

Another major cause of gastric ulcers is the use of Non Steroidal Anti-Inflammatory Drugs (NSAIDs). The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by some prostaglandins. NSAIDs block the function of cyclooxygenase 1 (COX-1), which is essential for the production of these prostaglandins. COX-2 selective anti-inflammatories (such as celecoxib) preferentially inhibit COX-2, which is less essential in the gastric mucosa, and roughly halve the risk of NSAID-related gastric ulceration. As the prevalence of H. pylori-caused ulceration declines due to increased medical treatment, a greater proportion of ulcers will be due to increasing NSAID use among individuals with pain syndromes as well as the growth of aging populations that develop arthritis (Johannessen, 2007). Gastrinomas (Zollinger Ellison syndrome), rare gastrin-secreting tumors, also cause multiple ulcers that are difficult to heal.

An expert panel convened by the Academy of Behavioral Medicine Research concluded that ulcers are not purely an infectious disease and that psychological factors do play a significant role (Peptic Ulcer, 2007). A study of peptic ulcer

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patients in a Thai hospital showed that chronic stress was strongly associated with an increased risk of peptic ulcer, and a combination of chronic stress and irregular mealtimes was a significant risk factor (Wachirawat *et al,* 2003).

Though the body system is made in such a way that it tackles invading foreign substances in most cases, the body system is incapable to do so and needs to be protected, enhanced and activated. This ability to activate the body defense mechanism or to protect the body system has been found to be present in some natural vegetation/herbal sources. And so it has become expedient to examine scientifically the protective effects of these herbal plants.

The plant extract of *tetracera potatoria* has been reported to possess anti-ulcer and anti-fungal activities (Adekunle *et al*, 2003; Adesanwo *et al*, 2003). One of the constituent chemicals found in *tetracera potatoria* is Betulinic acid (Adesanwo *et al*, 2003). The chemical screening of *vassourinha* plant extract used in herbal medicine in North America has shown the presence of scopadulcic acid A, B and C, scopadiol, scopadulciol, scopadulin, scoparic acids A, B and C, and Betulinic acid. It has demonstrated anti- inflammatory, anti-spasmodic and pain relieving activity in animal studies with rats, mice and guinea pig. In other in-vitro laboratory test, *vassourinha* demonstrated anti-oxidant actions as well as active antimicrobial properties (Freire *et al*, 1993; Freire *et al*, 1996). *Bacopa monnieri* is a plant that has been used for centuries in India as a tonic for the cardiovascular,

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digestive and respiratory systems. Some of the recently discovered constituents are betulinic acid and beta sitosterol. *Bacopa monnieri* has been found to prevent ulceration by various offensive and defensive mechanisms. It augments defensive mucosal factors like increased mucus secretion, life span of mucus membrane cell and gastric oxidation effects. In in-vitro studies, it showed anti-helicobacter pylori activity, increased prostanoid (PGE_2, PGI_2), and direct anti-spasmodic activity on smooth muscles of the intestinal and respiratory tract. In recent study, *bacopa monnieri* causes an increase in the production of mucin that provides protective barrier to the stomach lining. Other properties are anti-fungal, anti-microbial, stabilization of mast cells, scavenging of free radicals and protective effects on DNA via its free radical scavenging activity (Rao *et al*, 2000; Sairam *et al*, 2001). One of the most widely reported sources of betulinic acid is the birch tree (Betula spp., Betulaceae) where both betulinic acid and betulin can be obtained in substantial quantities (O'Connell *et al*, 1988; Cole *et al,* 1991; Galgon *et al*, 1999). Other known sources include Ziziphus spp. (Rhamnaceae), (Pisha *et al*, 1995; Schuhly *et al*, 1999; Jagadeesh *et al,* 1998), Syzygium spp. (Myrtaceae) (Kashiwada *et al,* 1998; Chang *et al,* 1999), Diospyros spp. (Ebenaceae)(Recio *et al,* 1995; Higa *et al,* 1998; Singh *et al,* 1997), and Paeonia spp. (Paeoniaceae) (Ikuta *et al,* 1995; Lin *et al,* 1998; Kamiya *et al,* 1997). Furthermore, given the widespread occurrence of the structurally related precursor betulin among plants, it

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is conceivable that the distribution of betulinic acid is even much greater (Hayek *et al.* 1989).

Thus betulinic acid has been identified and isolated in various plant species used in ethnomedicine across the world. The lupane-type triterpene betulinic acid is found widely throughout the plant kingdom. Hundreds of published reports have described the occurrence of betulinic acid across a multitude of taxonomically diverse genera. Betulinic acid exerts a number of biological activities. To this end, it is interesting to note that white birch bark (Betula alba) which contains betulinic acid, has been used by Native Americans as a folk remedy. Betulinic acid exerts a number of biological activities and has been found to possess the following properties: anti-retroviral (Mayaux *et al*, 1994; Evers *et al*, 1996), anti-tumor (Pisha *et al*, 1995; Schmidt *et al*, 2007), anti-ulcer (Adesanwo *et al*, 2003; Flekter *et al*, 2005), anti-bacteria (Pisha *et al*, 1995; Setzer *et al*, 2000).

This present study is thus to investigate the possible mode of action of betulinic acid in relation to its anti- gastric ulcer effect.

GENERAL OBJECTIVE

To evaluate the possible mechanism of anti-gastric ulcer effect of betulinic acid in male albino rats.

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SPECIFIC OBJECTIVES

1. To determine the effect of Betulinic Acid (BA) on indomethacin- induced gastric ulceration.
2. To determine the effect of Betulinic acid on gastric mucus secretion.
3. To determine the effect of Betulinic acid on gastric mucus cell count.
4. To determine the effect of Betulinic acid on basal and histamine-stimulated gastric acid secretion.
5. To determine the possible anti-oxidant effect of Betulinic acid through determination of Malonialdehyde (MDA) concentration.
6. To determine the histological changes due to the effect of Betulinic acid on gastric mucosal cells.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE STOMACH

The stomach is widely known for its role in food storage, processing and gastric acid secretion (Hersey and Sach, 1995). The stomach, located in the left upper quadrant of the abdomen just below the diaphragm, is a distensible saclike structure with strong, muscular walls. Arterial blood supply to the stomach is from the celiac trunk via the left and the right gastric artery while the venous drainage is through the left and right gastric veins into the portal vein. The innervations of the stomach involve the sympathetic nerve supply from T_6 - T_9 segments of the spinal cord. This passes to the celiac plexus through the greater splanchnic nerve. While the parasympathetic nerve supply is from the anterior vagus nerve (Keith Moore, 2000).

The stomach can expand significantly to store all the food from a meal for both mechanical and chemical processing. The stomach contracts about three times per minute, churning the food and mixing it with gastric juice (Fig. 2.1).

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The stomach consists of various glands which in turn consist of various cells responsible for secreting different materials. The oxyntic gland located mainly on the body of the stomach contains parietal cells which secrete hydrochloric acid and intrinsic factor and chief cells which secrete pepsinogen (a precursor to pepsin). The surface mucosal cells and the neck cells of the gastric gland secrete mucus and little bicarbonate (HCO_3^-). This fluid, secreted by thousands of gastric glands in the lining of the stomach, consists of water, hydrochloric acid, an enzyme called pepsin, and mucin (the main component of mucus). The mucus and the HCO_3^- play important roles in ensuring that the mucosa/lining of the stomach is not damaged by excess acidity. Changes in intra-gastric pH are a very important signal in the regulation of gastric acid secretion during meal. This is because gastric acid secretion is activated by the presence of food buffers which causes high luminal pH in the stomach.

Hydrochloric acid creates the acidic environment required for pepsin to begin the digestion of proteins. It also kills microorganisms that may have been ingested along with the food. Mucin coats the stomach, protecting it from the effects of the acid and pepsin. About four hours or less after a meal, food processed by the stomach, called chyme, begins passing a little at a time through the pyloric sphincter into the duodenum, the first portion of the small intestine.

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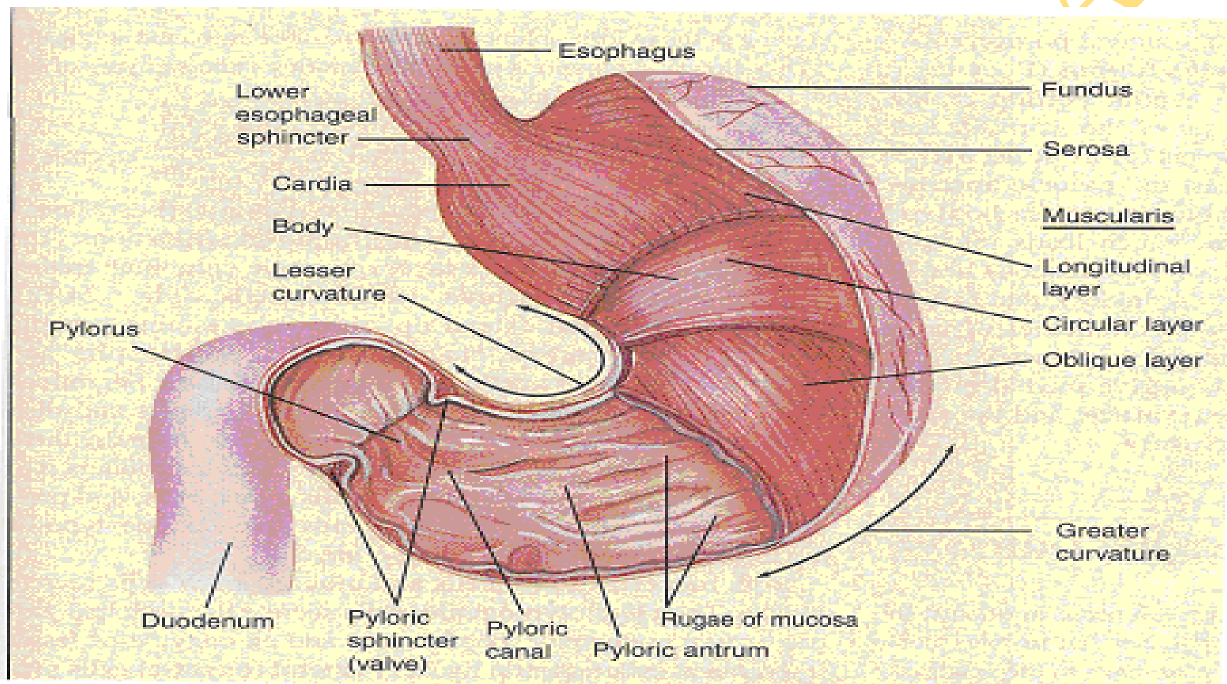

Fig 2.1: *Anatomical structure of the stomach.*

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2.2 HISTORY OF GASTRIC ACID

William Prout (1785-1850) is known for his discovery of the nature of the acid in the stomach of animals. He identified free hydrochloric acid in the gastric juice of various animals and humans after a meal and suggested that it was derived from the common salt of the blood by the force of galvanization (electricity). Before this finding, Prout favored phosphoric acid as the agent responsible for the acidity of gastric juice.

Army surgeon William Beaumont's (1785-1853) conducted a classical research on Alexis St. Martin, a Canadian with a permanent gastric fistula that remained after an accidental gunshot in 1822 had healed. Beaumont recognized the acid character of the gastric juice secreted in response to food and alcohol ingestion. He published his findings in *Experiments and Observations on Gastric Juice and the Physiology of Digestion* (1833).

All doubt was finally dispelled by the publication in 1852 of "Gastric juice and Metabolism: A Physiological-Chemical Investigation" by Fredrick Bidder (1810-1894) and Carl Schmidt (1822-1894) of the University of Dorpat. From their quantitative analyses of the gastric juice collected by means of a fistula created in different species of live animals, Bidder and Schmidt proved that the acid of gastric juice is exclusively hydrochloric acid.

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In 1878, Heindehain removed a small portion of the greater curvature of the stomach and formed it into a pouch. The secretion of the pouch could be removed through a fistula made by bringing the opening of the pouch through a stab wound in the belly wall. A pouch such as this may be considered to represent a miniature stomach, which mirrors the secretory events occurring in the stomach. The secretion obtained from the pouch were not contaminated with food, saliva or materials regurgitated from the intestine. The vagal connection to the Heindahain pouch were completely severed, therefore, results obtained from this preparation may not be representative of secretion as it occurs in the main stomach.

However, the pouch is useful when gastric secretion is to be studied in the absence of vagal innervations (Murphy, 1998). In 1902, Pavlov Jan Petrovitch made a pouch to which many vagal connections were maintained intact. Continuity between the pouch and the main stomach was retained by a bridge of tissue through which vagal nerve fibers traveled to the pouch. Thus, the gastric secretions were obtained (Murphy, 1998).

Another was an animal with esophagotomy. Since ingested materials can be drained from the upper esophageal fistula, food can be administered orally without coming into contact with the more distal regions of the digestive tract. On the other hand, food can be introduced through the lower esophageal fistula in order to eliminate stimulation of the oral cavity. As such gastric juice is obtained. However,

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a gastric pouch in conjunction with esophagotomy is often used in order to obtain gastric juice (Murphy, 1998).

Hollandes (1954) first differentiated gastric secretions as parietal and non-parietal secretions. Later this non-parietal secretion was identified as bicarbonate secretion (Allen *et al,* 1993).

2.3 METHODS OF STUDYING GASTRIC ACID SECRETION

For more than a century, not much was done to investigate the amount of gastric acid secreted because of the unavailability of a device that can collect pure gastric juice without contamination with food particles. In animals, it is possible to obtain samples of juice secreted only in the parietal cells by isolating a pouch of the mucosa of the stomach. The collection of gastric juice from pouches was first devised by Pavlov (1910). He did this by making an incompletely separated pouch of part of the stomach and ensured that the vagus nerve was intact. Thus, the pouch was referred to as 'vagally' innervated pouch.

In recent times, in vivo and in vitro methods have been devised to study gastric acid secretion. In vitro methods were used by Davies (1948) and Davenport (1957). They used the method to describe the membrane transport across the gastric mucosa and several other chemical events.

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In vivo studies involve the use of intact, whole and conscious animals. The methods include: Nasogastric tube, gastric fistula, continuous perfusion of the stomach, gastric pouches, sham feeding.

Nasogastric tube: An aspirating in-dwelling tube is inserted into the stomach through the mouth or nose. The tube is placed in the most distended portion of the stomach of the animal under fluoroscopy. Through this tube, gastric secretion can be collected and analyzed for volume, pH concentration of HCl, pepsin, etc.

Gastric fistula: Procedure was first used by Beaumont (1833) on a child who sustained a gunshot wound and it was feared that the gastric function would be seriously endangered as the bullet hit the stomach directly. In order to help the child a gastric fistula was placed in the body of the stomach very close to the antrum along the greater curvature to provide for optimal drainage and diversion of acid from the antral and duodenal mucosa. This helped to normalize gastric function. Other scientists such as Basou (1842) and Blondot (1843) used this procedure on dogs.

Continuous perfusion of the stomach: This method was devised by Ghosch and Schild (1958) by using a perfusate which changes its pH when acid is secreted. The animal to be used is fasted at least 24 hrs prior to the time of the experiment. The animal is anaesthetized using intra – peritoneal injection of urethane (25%) at a

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dosage of 0.6 ml / 100g of animal body weight. The animal is then laid supine on a board and its limbs are tied to prevent movement.

The fur in the neck region is shaved, the underlying connective tissue is cut open by blunt dissection to minimize bleeding, and this exposes the trachea. A small incision is made on the upper part of the trachea to ensure free flow of air and increase ventilation.

The fur in the middle part of the linea alba is shaved and a small midline incision is made into the abdominal muscle. The underlying connective tissue and fascia are both removed using blunt dissection. When the stomach is identified, a small incision is made at the gastro-duodenal junction and a cannula is inserted and ligated with a thread.

The esophageal cannula of the Watson Marlow's flow meter or the modified Langerdoff's apparatus is inserted through the mouth of the animal. Perfusing fluid which is normal saline is then run through the esophageal cannula into the stomach and out through the duodenal cannula. This is done to wash out any remaining debris and avoid contamination of the gastric juice. The stomach is returned into the peritoneum with the cannula still in place and collection of effluent is done every 10 minutes.

Gastric pouch preparation: This method allows for direct investigation into the functions of the stomach. The first gastric pouch was developed by Pavlov (1910).

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Other scientists have since modified the Pavlov's pouches (Katch, 1912; Ivy, 1926 and Heidehain, 1987). These pouches help to investigate both the neural and hormonal control of the secretory functions of the stomach by separating these controls and observing their individual effects on gastric acid secretion.

Sham feeding: This involves stimulating the cephalic phase of gastric secretion by stimuli such as smell or taste of food. This method was used by Pavlov to study the post-prandial response of the stomach to food intake. An esophageal opening is created so that all swallowed food will go in through this opening. A cannula is then placed in the stomach to collect gastric acid secretion (Best and Taylor, 1990).

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2.4 MAJOR COMPONENTS OF GASTRIC JUICE

2.4.1 *ROLE OF PARIETAL CELLS IN GASTRIC ACID SECRETION*

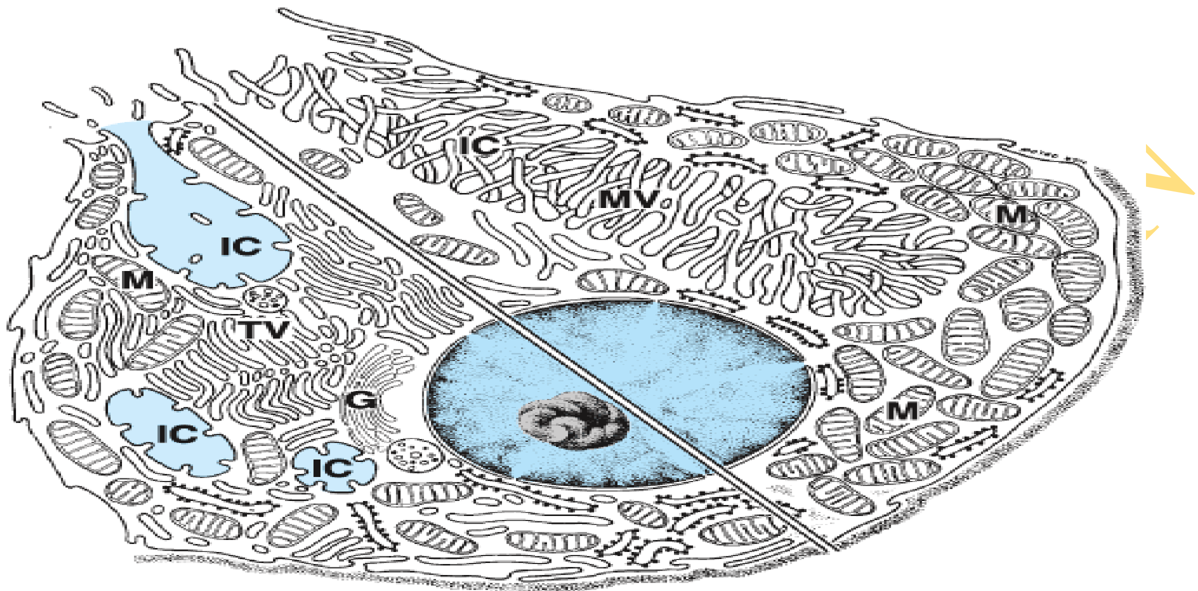

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Fig.2.2: *Composite diagram of a parietal cell, showing the resting state (lower left) and the active state (upper right). The resting cell has intracellular canaliculi (IC), which open on the apical membrane of the cell, and many tubulovesicular structures (TV) in the cytoplasm. When the cell is activated, the TVs fuse with the cell membrane and microvilli (MV) project into the canaliculi, so the area of cell membrane in contact with gastric lumen is greatly increased mitochondrion M, and Golgi apparatus G.*

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In humans, the rate of secretion of gastric acid is about 2 to 3 liters per day (Guyton and Hall, 2000). Chemically, gastric acid consists mainly of hydrochloric acid (HCl) (around 0.5%, or 5000 parts per million), and large quantities of potassium chloride (KCl) and sodium chloride (NaCl). The secretion of gastric juice by the mammalian stomach mucosa cells in the intact animal is under both neural and hormonal control. It is influenced by the interplay of stimulatory and inhibitory arising from both the central nervous system and within the gastrointestinal system (Xuebaio and Forte, 2003).Gastric acid is one of the main isotonic solutions secreted and together with several enzymes and intrinsic factors in a heterogeneous solution is called gastric juice (Fig. 2.2).

Gastric acid is produced by parietal cells (also called oxyntic cells) in the stomach. Its secretion is a complex and relatively energetically expensive process. Parietal cells contain an extensive secretory network (called canaliculi) from which the gastric acid is secreted into the lumen of the stomach. These cells are part of epithelial fundic glands in the gastric mucosa. The pH of gastric acid is 1.8 to 3.5 in the human stomach lumen, the acidity being maintained by the proton pump H^+/K^+ ATPase.

The parietal cell releases bicarbonate into the blood stream in the process, which causes the temporary rise in pH in the blood, known as alkaline tide.

Parietal cells that secrete the gastric acid have 4 major receptors namely:

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1. H_2 receptors that respond to histamine from the ECL cells
2. Muscarinic receptors that responds to acetylcholine from the vagus nerve
3. SST_2 receptors that respond to somatostatin from D cells
4. CCK_2 receptors that respond to gastrin from the G cells

The diagram below shows schematically the process of gastric acid formation and secretion. The hydrochloric acid is formed at the villus-like projections inside these canaliculi and is then conducted through these canaliculi to the exterior.

Early investigations defined most of our knowledge about gastric chloride secretion but are derived solely from in vivo (steady state) experiments or in vitro preparations of isolated amphibian mucosa. More recent information from mammalian systems have identified baso-lateral Na^+-K^+-Cl^- co-transporter NKCC1 and Cl^-/HCO_3^- exchanger AE2 as potential routes for chloride uptake by gastric epithelial cells and has identified a CLC-2 as a pH sensitive Cl^- channel that may represent an apical efflux route for Cl^- secretion in parietal cells. The principle of electrical neutrality of solutions requires that exactly the same number of anions as hydrogen ions be present. In order to satisfy this principle, chloride ions pass from the plasma through the cells into the secretion being dragged along by the positive charge of the H^+. The chloride ions are replaced by bicarbonate ions formed in the cells alongside hydrogen ions (Bulger *et al*, 1928).

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2.4.2 *MECHANISM OF HCL SECRETION BY PARIETAL CELL IN THE STOMACH*

The most recent theory of the mechanism of gastric acid secretion is the H^+-K^+-ATPase theory {Davies (1948) and Davenport (1957)}. Chloride ions are actively transported from the cytoplasm of the parietal cell into the lumen of the canaliculus and sodium ions are actively transported out of the lumen. A potential of about -40 to -70 mV is created in the canaliculus. This negative potential causes the diffusion of positively charged potassium ions and a small amount of sodium ions from the cell cytoplasm into the canaliculus (Fig. 2.3).

Water dissociates into H^+ and OH^- in the cell cytoplasm. The hydrogen ions are actively secreted into the canaliculus in exchange for potassium ions and this exchange is catalyzed by H^+-K^+-ATPase. In addition, the sodium ions are actively reabsorbed by a separate sodium pump. Thus most of the sodium and potassium ions that entered into the canaliculus are reabsorbed into the cell cytoplasm and hydrogen ions take their place in the canaliculus giving a strong solution of hydrochloric acid which is secreted into the lumen of the oxyntic gland (Fig. 2.3).

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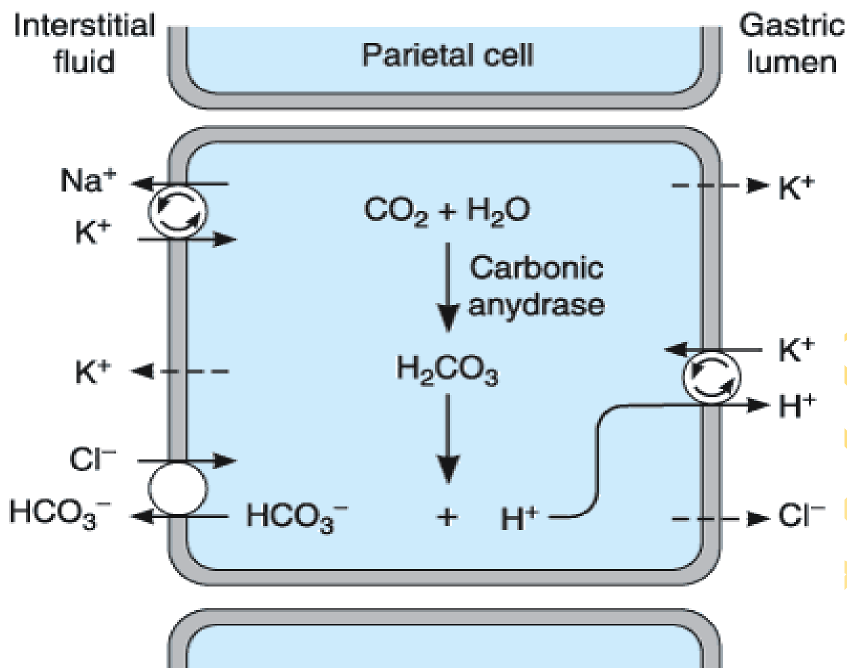

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Fig. 2.3: *HCl secretion by parietal cells in the stomach. Active transport by ATPase is indicated by arrows in circles.* H^+ *is secreted into the gastric lumen in exchange for* K^+ *by* H^+*–*K^+ *ATPase.* HCO_3^- *is exchanged for* Cl^- *in the interstitial fluid by an anti-port, and* Na^+*–*K^+ *ATPase keeps intracellular* Na^+ *low. Dashed arrows indicate diffusion.*

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Water passes into the canaliculus by osmosis due to the secretion of osmotically active ions into the canaliculus. Thus, the final secretion from the canaliculus contains hydrochloric acid at an approximate concentration of 150 to 160 mEq/L, potassium chloride at a concentration of 15mEq/L and a small amount of sodium chloride. The enzyme called carbonic anhydrase catalyses the reaction between carbon dioxide formed during cellular metabolism or entering the cell from blood and water to form carbonic acid. This acid immediately dissociates into hydrogen and bicarbonate ions. The hydrogen ions leave the cell through H^+/K^+ ATPase anti-port pumps while the bicarbonate ions diffuse out of the cell cytoplasm into extracellular fluid in exchange for chloride ions. The highest concentration that it reaches in the stomach is 160 mM per liter in the canaliculi. This is about 3 million times that of arterial blood, but almost exactly isotonic with other body fluids. The lowest pH of the secreted acid is 0.8 demonstrating its extreme acidity, but the acid is diluted in the stomach lumen to a pH between 1 and 3. To concentrate this tremendous amount of H^+ more than 1500 calories of energy is required per liter of gastric juice.

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2.4.3 *PHASES OF GASTRIC ACID SECRETION*

There are three phases in the secretion of gastric acid:

1. The cephalic phase: 30% of the total gastric acid to be produced is stimulated by anticipation of eating and the smell or taste of food. This phase is controlled by the brain and may be stimulated by thoughts. Vagal impulses cause the release of acetylcholine in the body of the stomach. Ach both directly increase gastric acid secretion and also stimulate histamine release.

2. The gastric phase: 60% of the acid secreted is stimulated by the distention of the stomach with food and protein digestion, which causes even more gastrin production. Also low pH at the antral portion of the stomach inhibits this phase through the release of somatostatin which is an important control mechanism in the regulation of gastric acid secretion.

3. The intestinal phase: the remaining 10% of acid is secreted when chyme enters the small intestine, and is stimulated by small intestine distention.

2.4.4 *REGULATION OF GASTRIC ACID SECRETION*

Central regulation of gastric acid secretion involves cortical and spinal cord structures which change or alters the balance between the parasympathetic and sympathetic outflow of the myenteric plexus in the gastric wall (Tache, 1987).

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Parasympathetic stimulation to the stomach leads to increased gastric acid secretion.

Gastric acid production is regulated by both the autonomic nervous system and several hormones. The parasympathetic nervous system via the vagus nerve and the hormone gastrin stimulate the parietal cell to produce gastric acid, both directly acting on parietal cells and indirectly, through the stimulation of the secretion of the hormone histamine from enterochromaffin-like cells (ECL). Vasoactive intestinal peptide, cholecystokinin, and secretin all inhibit gastric acid production.

The production of gastric acid in the stomach is tightly regulated by positive and negative feedback mechanisms. Four types of cells are involved in this process: parietal cells, G cells (Buchan, 1991), Delta cells and enterochromaffin-like cells (Hakanson, 1967; Prinz, 1993). Besides this, the endings of the vagus nerve (X) and the intramural nervous plexus in the digestive tract influence the secretion significantly.

Nerve endings in the stomach secrete two stimulatory neurotransmitters: acetylcholine and gastrin-releasing peptide (Wood, 1994). Their action is both direct on parietal cells and mediated through the secretion of gastrin from G cells and histamine from enterochromaffin-like cells. Gastrin acts on parietal cells directly and indirectly, by stimulating the release of histamine (Prinz, 1994).

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Hormonal factors also play a very important role in gastric acid secretion. The release of histamine is the most important positive regulatory mechanism of the secretion of gastric acid in the stomach. Its release is stimulated by gastrin and acetylcholine and inhibited by somatostatin (Berglingh, 1976).

(a) Histamine

Histamine is a biogenic amine involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter. Histamine is synthesized from the decarboxylation of the amino acid histidine, a reaction catalyzed by the enzyme L-histidine decarboxylase (L-HDC). It is a hydrophilic vasoactive amine and stored in mast cells, enterochromaffin – like (ECL) cells and enteric nerve fibers in the stomach. Histamine released from the ECL cells stimulates gastric acid secretion via H_2 receptors located on parietal cells in the stomach mucosa by increasing intra-cellular cyclic Adenosine Mono Phosphate ($_C$AMP). However, it has been shown recently that histamine elevates intra-cellular calcium in parietal cells suggesting that this receptor has at least a dual coupling system in this cell type (Chew, 1986).

H_2 antagonists are also inverse agonists and not true antagonists. H_2 histamine receptors are found principally in the parietal cells of the gastric mucosa. H_2 antagonists are used to reduce the secretion of gastric acid, treating gastrointestinal

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conditions including peptic ulcers and gastro-esophageal reflux disease. Examples include cimetidine and famotidine.

(b) Acetylcholine

Acetylcholine (Ach) is a neurotransmitter and secretagogue released by all secretory nerves. Acetylcholine was first identified in the year 1914 by Henry Hallett Dale for its actions on heart tissue. It was confirmed as a neurotransmitter by Otto Loewi who initially gave it the name vagusstoff because it was released from the vagus nerve. Both received the 1936 Nobel Prize in Physiology or Medicine for their work. Acetylcholine was also the first neurotransmitter to be identified. Acetylcholine is an ester of acetic acid and choline with chemical formula $CH_3COOCH_2CH_2N^+(CH_3)_3$. This structure is reflected in the systematic name, *2-acetoxy-N,N,N-trimethylethanaminium*. Acetylcholine acts directly on the parietal cells by binding to M_3 muscarinic receptors which in turn lead to increase in intra-cellular Ca^{2+} presumably coupled to a Gq trimeric protein (Pfeifer, 1990; Wilkes, 1991; Ganong, 2005).

The mechanism underlying the regulation of gastric acid secretion from parietal cells by Ach involves two pathways. The first pathway involves direct activation of M_3 receptors on parietal cells as evidenced by the fact that carbachol enhanced gastric acid secretion in HDC-KO mice in which histamine release from ECL cells

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is absent. The second pathway involves indirect stimulation of parietal cells via release of hormones or transmitters from endocrine cells. In particular, in vivo histamine release from ECL cells have been reported to be enhanced by cholinergic stimulation. In a study in WT mice, carbachol increased gastric histamine synthesis and secretion which was completely inhibited by atropine, whereas famotidine significantly inhibited carbachol – stimulated acid secretion in WT mice. Such result indicates that histamine secretion from ECL cells is evoked by cholinergic stimulation via mAchR's activation.

(c) Gastrin

Gastrin released from G cells has been reported to be directly stimulated by acetylcholine via muscarinic receptors M_3 receptor-mediated gastrin might be involved in carbachol stimulated histamine secretion from ECL cells. Gastrin is the major stimulatory endocrine peptide for histamine release from ECL (Prinz, 1994). It is secreted by G-cells of the antral mucosa of the stomach. It also acts by stimulating the secretion of histamine from ECL cells. Gastrin binds to CCk – B receptors present on ECL cells and parietal cells, releasing histamine by increasing intracellular free Ca^{2+} (Ganong, 2005).

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INHIBITORY MECHANISMS

A variety of substances are known to inhibit gastric secretion when they come in contact with the duodenal mucosa (examples are fat digestion products, acids and hypertonic solutions). When food is ingested, gastric secretion is stimulated by a variety of mechanisms. Nevertheless, there are also inhibitory mechanisms, the function of which is to prevent excessive secretion (Sachs *et al*, 1994).

If liver solution at a pH of 7 is introduced into an antral pouch, it is denervated and secretes acid. On the other hand, if the pH of liver solution is 2, no response is obtained. It is apparent that acid in contact with the antral mucosa inhibits secretion. Thus, inhibition is proportional to the hydrogen ion concentration (H^+) in the content of the antrum. Although, it has been suggested that inhibition of acid secretion is as a result of suppression of the release of gastrin.

For example, the gastric secretions that are inhibited in bathing the antral mucosa with acid are known to be elicited especially by the stimuli that release gastrin. On the other hand, there is no inhibition for stimuli that operate by means other than gastrin release. The mechanism by which acid suppresses gastrin release is unknown. However, there is no application of anesthetics to the antral mucosa. Acid must therefore manifest its inhibitory effect distal to the site of acetylcholine release, i.e. the gastrin – releases cells may be sensitive to hydrogen ion (Ewald, 1976).

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An auto regulatory mechanism of the antral content is low at different times of the day; this mechanism may play an important role in regulating gastric acid secretion. For example, during inter-digestive periods, the pH of the stomach content is low and the release of gastrin is suppressed. When a meal is eaten, the acid which is present is buffered by the constituent of the food, antral pH rises, the inhibition is removed, and gastrin is released in respond to the usual stimuli. Gastric juice is secreted at a high rate which continues until the buffering power of whatever food remains in the stomach is exhausted. At this time, pH decreases and the resultant acidification of the antrum brings the gastric phase to an end (Ewald, 1976).

Although, the effect of intravenous administration of cholecystokinin do eliminate gastric acid secretion, the actual result of liberations of this hormone from the intestinal mucosa in response to a meal, may be one of inhibition. The possibility is based on that there is competitive inhibition of gastrin by cholecystokinin in the process of gastric secretion of acid. Since gastrin and cholecystokinin possess the same terminal tetra peptide, it is reasoned that these two molecules compete for the same acid stimulating receptor sites on the parietal cells.

However, cholecystokinin is a weak stimulant compared to gastrin. When both hormones are released in response to a meal, the overall gastric acid secretory response would be less than that of gastrin alone because gastrin is unable to

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manifest its full excitatory effects in the presence of cholecystokinin i.e. cholecystokinin denies receptor sites of gastrin (Thompson *et al*, 1987).

(d) Somatostatin

A low pH stimulates the release of somatostatin. Somatostatin is a known inhibitor of gastric acid secretion. It is D cell that synthesizes and secrets somatostatin in mammals. D cell is located near G cell in gastric antrum, and along gastric gland, particularly near the parietal cell in oxyntic mucosa. Somatostatin, mediated by its receptor in the membrane of parietal cells inhibits gastric acid secretion induced by gastrin and acetylcholine. In parietal cells, somatostatin receptors are coupled to adenyl cyclase via an inhibitory guanine nucleotide binding protein. In addition, somatostatin inhibits the gastrin secretion in the basal condition or the gastrin secretion induced by feeding, acetylcholine and bombesin (Zhang Zhi-Fang *et al*, 1998). In stomach, somatostatin inhibits the histamine secretion in the basal condition or induced by gastrin. It inhibits histamine – stimulated adenosine 3' -5'-cyclic monophosphate (cAMP) production and aminopyrine accumulation, an index of acid production.

Pharmacologically, several drugs have been used to inhibit the production of gastric acid secretion. Their mechanism of action is based on the fact that they block receptors that are found on the parietal cells. Examples of them include:

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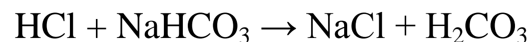
- Histamine H_2 receptor antagonists: cimetidine, ranitidine, famotidine, metiamide and burimamide.
- Muscarinic M_3 receptor antagonists: atropine.
- gastrin CCK_2 receptors antagonists include: YF476, YM022, RPF3870, JB93182, AGO41R and L-365,260
- Proton-pump inhibitors: omeprazole, lansoprazole and pantoprazole

2.5 NEUTRALIZATION OF GASTRIC ACID

In the duodenum, gastric acid is neutralized by sodium bicarbonate. This also blocks gastric enzymes that have their optima in the acid range of pH. The secretion of sodium bicarbonate from the pancreas is stimulated by secretin. This polypeptide hormone gets activated and secreted from so-called S cells in the mucosa of the duodenum and jejunum when the pH in duodenum falls below 4.5 to 5.0. The neutralization is described by the equation:



$$HCl + NaHCO_3 \rightarrow NaCl + H_2CO_3$$

The carbonic acid instantly decomposes into carbon dioxide and water, and then gets eliminated through urine (Best and Taylor, 1990).

Prostaglandins have been noted to form a vital component of the gastric mucosal defense as they are known to have an anti-secretory effect on gastric acid. They are

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formed throughout the gut and the major stimulant for their synthesis is cell trauma. They are also known to stimulate the synthesis of mucus. Alkalinity of the *lamina propia*, rate and quality of the mucus secreted, adequacy of mucosal blood flow and the rate at which the gastric epithelium replaces itself are all part of the mucoprotective factors which protects the mucosal prostaglandins (Dwork, 1982).

2.6 FUNCTIONS OF THE COMPONENTS OF GASTRIC JUICE

Hydrochloric Acid

Hydrochloric acid secreted from parietal cells into the lumen of the stomach performs the following:

It establishes an extremely acidic environment in the stomach.

It helps in the activation of pepsinogen to pepsin.

It aids the destruction of microorganism ingested along with food.

It provides an optimal low pH for peptic digestion.

It is essential in the gastric breakdown of connective tissue and muscle fiber (Ribbon *et al,* 1990).

Pepsinogen and Pepsin

Pepsinogen secreted as inactive zymogens into the gastric juice from both mucous and chief cell is activated by hydrochloric acid into active protease pepsin.

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Pepsin is largely responsible for the stomach ability to initiate digestion of proteins. Pepsin in young animals as chymosin helps in the coagulation of milk protein allowing it to be retained more than briefly in the stomach (James *et al*, 1996).

Mucus

The bicarbonate-rich mucus secretes into the glands as mucous neck cell:

a. Coats and lubricates the gastric surface

b. Protects the epithelium from acid and other chemical insults.

c. Aids barrier function via gastric mucosal barrier.

4. Maintains neutral pH of epithelium plasma membrane (Engle *et al*, 1995)

Gastrin

The principal hormone secreted from the gastric epithelium in the form of peptide helps in:

a. Control of acid secretion.

b. Control of gastric motility (Walsh, 1993).

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Intrinsic Factor

A glycoprotein of considerable importance secreted by the parietal cells and is necessary for intestinal absorption of Vitamin B_{12} and formation of normal red blood cells (erythrocytes) (Toh *et al*, 1997).

2.7 EFFECTS OF UNDER SECRETION OF GASTRIC JUICE

(i) Gastric Atrophy

In many people with chronic gastritis, the mucosa gradually becomes atrophic until little or no gastric gland activity remains. It is also believed that some people develop auto immunity against the gastric mucosa eventually leading to gastric atrophy. Hence, there is decreased gastric motility (Guyton *et al*, 2000).

(ii) Achlorhydria (and Hypochlorhydria)

Achlorhydria refers to a condition whereby the stomach fails to secrete hydrochloric acid, even in its minute form. This is diagnosed when the pH of the gastric secretion fails to decrease below 6.5 after maximal stimulation such that when acid is not secreted pepsin activity is affected even when it is the lack of acid that prevents it from functioning as pepsin requires an acidic medium for its activity. While hypochlorhydria is a condition of diminished acid secretion (Guyton, et. al., 2000). However, the overall digestion of food in the entire gastro

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intestinal tract is still almost normal even with achlorhydria. Thus, the symptomology of achlorhydria is limited to the stomach (Guyton *et al*, 2000).

(iii) Pernicious Anaemia

In all mammals, Vitamin B_{12} is essential for maturation of erythrocytes and a deficiency of this vitamin leads to the development of anemia. Since the efficient absorption of vitamin B_{12} in man depends on intrinsic factor, diseases which decrease the secretion of intrinsic factor e.g. gastric atrophy and achlorhydria interfere with the cleavage of the binding proteins (i.e. in pancreatic exocrine deficiency) or decrease binding and absorption of intrinsic factor – vitamin B_{12} can result in pernicious anemia (Toh *et al*, 1997).

In addition, pernicious anemia frequently occurs after most of the stomach has been removed for treatment of gastric ulcers or when the terminal ileum where Vitamin B_{12} is almost entirely absorbed has been removed in the absence of intrinsic factor, about 1/50 of Vitamin B_{12} is not made available from the foods. As a result, young newly forming red blood cells fail to mature while they are still in the bone marrow (Toh *et al*, 1997).

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2.8 EFFECTS OF EXCESSIVE GASTRIC SECRETION

(i) Gastritis

This refers to the inflammation of the gastric mucosa. Mild to moderate chronic gastritis is exceedingly common in human population as a whole especially in the later year of adult age. The inflammation of gastritis may be either superficial or deep.

Superficial inflammation is not very harmful while deep inflammation may penetrate deeply into the gastric mucosa and cause almost complete atrophy of the gastric mucosa in long lasting cases. Research suggests that much gastritis is caused by chronic bacteria infection i.e. Helicobacter pylori of the gastric mucosa. Also, certain ingested irritant substances e.g. alcohol and aspirin can be especially damaging to the protective gastric mucosal barrier (i.e. the tight epithelial junctions and the mucous glands). Thus, permeability of the mucous barrier is greatly increased such that hydrogen ions (H^+) do then diffuse into the stomach epithelium creating an additional havoc leading to a vicious cycle of progressive stomach mucosal damage and atrophy. The mucosa also becomes susceptible to peptic digestion resulting in severe acute or chronic gastric ulcer in most cases (Walsh *et al*, 1995).

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(ii) Peptic Ulceration

An ulcer is a discontinuity of the mucosa surface of some part of the gastrointestinal tract (GIT) with an inflammatory base. A peptic ulcer, also known as ulcus pepticum, PUD or peptic ulcer disease, is an ulcer (defined as mucosal erosions equal to or greater than 0.5 cm) of an area of the gastrointestinal tract that is usually acidic and thus extremely painful (GI Consult, 2007). Ganong (1977) stated that peptic ulceration in humans is related primarily to a breakdown of barrier that normally prevent irritation and auto-digestion of the mucosa by the gastric secretions. In other words, gastric ulceration occurs due to an imbalance between the rate of secretion of gastric juice and the degree of protection afforded by the gastric juice as well as the neutralization of the gastric juice (Ganong and Hall, 1996).Thus, those effects which increases the production of acid and pepsin are termed ulcerogens while those factors that reduce their secretion are said to be mucoprotective.

Contrary to general belief, more peptic ulcers arise in the duodenum (first part of the small intestine, just after the stomach) than in the stomach. Duodenal ulcers are generally benign. The development of peptic ulcer is determined by the algebraic sum of defensive and aggressive forces acting on the gastrointestinal mucosa. Peptic ulcer will develop when the summation of these forces was resolved in favour of the aggressive factor, being the gastric hydrochloric acid, HCL

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(Hollander, 1954). The agents known to increase acid production are known to cause ulceration of the mucosa of the GIT (Gregory *et al*, 1967). Imbalance of acid secretion can also be due to blood group, Helicobacter pylori and some disease conditions such as cirrhosis, arthritis and hyperthyroidism (Aeird *et al*, 1953; Mc Quaid *et al*, 1992).

Classification

- Stomach (called gastric ulcer)
- Duodenum (called duodenal ulcer)
- Oesophagus (called Oesophageal ulcer)
- Meckel's Diverticulum (called Meckel's Diverticulum ulcer)

Types of peptic ulcers:

Type I: Ulcer along the lesser curve of stomach

Type II: Two ulcers present - one gastric, one duodenal

Type III: Prepyloric ulcer

Type IV: Proximal gastroesophageal ulcer

Type V: Anywhere along gastric body, NSAID induced

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Signs of peptic ulcers

A history of heartburn, gastroesophageal reflux disease (GERD) and use of certain forms of medication can raise the suspicion for peptic ulcer. Medicines associated with peptic ulcer include NSAID (non-steroid anti-inflammatory drugs) that inhibit cyclooxygenase, and most glucocorticoids (e.g. dexamethasone and prednisolone). The timing of the symptoms in relation to the meal may differentiate between gastric and duodenal ulcers. A gastric ulcer would give epigastric pain during the meal, as gastric acid is secreted, or after the meal, as the alkaline duodenal contents reflux into the stomach. Symptoms of duodenal ulcers would manifest mostly before the meal—when acid (production stimulated by hunger) is passed into the duodenum. However, this is not a reliable sign in clinical practice.

Symptoms of peptic ulcers

Symptoms of a peptic ulcer can be

- Abdominal pain, classically epigastric with severity relating to mealtimes, after around 3 hours of taking a meal (duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it);

- Bloating and abdominal fullness;

- Waterbrash (rush of saliva after an episode of regurgitation to dilute the acid in esophagus);

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- Nausea, and copious vomiting;

- Loss of appetite and weight loss;

- Hematemesis (vomiting of blood); this can occur due to bleeding directly from a gastric ulcer, or from damage to the esophagus from severe/continuing vomiting.

- Melena (tarry, foul-smelling feces due to oxidized iron from hemoglobin);

- Rarely, an ulcer can lead to a gastric or duodenal perforation. This is extremely painful and requires immediate surgery.

Complications of Peptic Ulcer

Gastrointestinal bleeding is the most common complication. Sudden large bleeding can be life-threatening (Cullen *et al.* 1997). It occurs when the ulcer erodes one of the blood vessels.

Perforation (a hole in the wall) often leads to catastrophic consequences. Erosion of the gastro-intestinal wall by the ulcer leads to spillage of stomach or intestinal content into the abdominal cavity. Perforation at the anterior surface of the stomach leads to acute peritonitis, initially chemical and later bacterial peritonitis. The first sign is often sudden intense abdominal pain. Posterior wall perforation leads to pancreatitis; pain in this situation often radiates to the back.

Penetration is when the ulcer continues into adjacent organs such as the liver and pancreas (Peptic Ulcer, 2007). Scarring and swelling due to ulcers causes

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narrowing in the duodenum and gastric outlet obstruction. Patient often presents with severe vomiting.

(iii) Indomethacin-Induced Gastric Ulceration

Indomethacin (1-p-chlorobenzoy-1,5 methoxy-2- methlindole-3-acetic acid) have anti-inflammatory and antipyretic effects. Local or parenteral administration of indomethacin to fasting rats induced acute superficial necrotic lesions in the gastric mucosa (Roberts, 1977). Administration of 40mg/kg BW of indomethacin in rats induces edema, cellular necrosis and haemorrhageal of parts of the gross abdominal feature (Elegbe *et al*, 1988). Indomethacin exerts its effect by decreasing the rate of secretion of gastrointestinal mucus and the lowering of the concentration of the component of the gastric mucosubstance (Menguy and Desbaillet, 1967). Reduction of the mucus secretion will expose the mucosa lining of the GIT to the action of acid peptic secretion resulting in lesions. Indomethacin like other non-steroidal anti-inflammatory drugs inhibits the biosynthesis of prostaglandin (Vane, 1971). While Main and Whittle (1975) reported that the ulcerogenic effect of indomethacin is due to its causing local trauma of the blood vessels supplying the gastric mucosa, thus predisposing to a decrease in gastric mucosal blood flow.

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Ulcer Scoring

Ulceration in the stomach can be assessed by means of a scoring technique. To achieve this, the stomach has to be exposed surgically and opened up by an incision along the lesser curvature. A macroscopic examination of the stomach can then be made using a magnifying hand lens.

Alphin and Wards (1968) used a scoring technique that has to do with easy handling of low figures. In this method, ulcer score ranges from 0 - 3.0 and are as follow:

Score	Interpretation
0	Normal Stomach
0.5	Gray discolouration and thinning of mucosa
1.0	Pin-point ulcer
2.0	One or two small ulcers
3.0	Several ulcers.

Elegbe and Bamgbose (1976) used two different scoring methods which were modifications for the method of Alphin and Wards (1968) to assess the ulceration produced by starvation and indomethacin. They suggested that the method of scoring ulcer depends on the methods of induction of the ulcer since ulcerogenic

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agents produce ulcers in varying degrees. With indomethacin induced ulceration, the following criteria were use:

Score	Interpretation
0	Normal Stomachs
0.5	Punctuate haemorrhage or pin point ulcers
1.0	Two or more small haemorrhagic ulcers
2.0	Ulcer greater than 3mm in diameter

In this method, a score of less than 1.0 was taken as an offer of protection.

2.9 TREATMENT OF ULCER

The treatment of chronic peptic ulcer involves the treatment of acute exacerbation and the implications and prevention of ulcer reoccurrence. The long term management of ulcer involves the use of drugs aimed at inhibiting acid secretion and enhancing mucosal resistance to acid and pepsin. Drugs employed for long term management of peptic ulcer include anticholinergic drugs, H_2- receptor antagonist and prostaglandins (PgA_2, PgB_1 and PgB_2). Surgery is carried out through the proximal gastric vagotomy for duodenal ulcer. If this fails, antrectomy is usually employed.

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2.9.1 ULCER HEALING

Ulcer healing , a genetically programmed repair process includes inflammation, cell proliferation, re-epithelization, formation of granulation tissue, angiogenesis, interactions between various cells and the matrix and tissue modeling, all resulting in scar formation. All these events are controlled by the cytokines growth factors (EGF, PDGF, KGF, HGF, TGF_{beta}, VEGF, angiopoietins) and transcription factors activated by tissue injury in spatially and temporally coordinated manner. This growth factors trigger mitogenic and survival pathways utilizing Ras, MAPK, PI-3K/AKT, PLC-gamma and Rho/Rac/actin signaling. Hypoxia activates proangiogenic genes (e.g VEGF angiopoietins) via HIF, while serum response factor (SRF) is critical for VEGF-induced angiogenesis, re-epithelialization and muscle restoration. EGF, its receptors HGF and COX-2 are important for epithelial cell proliferation, migration re-epithelialization and reconstruction of gastric glands. VEGF, Angiopoietins, nitric oxide, endothelin and mucosal regeneration within ulcer scar (Wallace *et al,*2006). Circulating progenitor cells are also important for ulcer healing.

Cyclooxgenase (COX) is an enzyme that is responsible for the formation of prostanoids. The three main groups of prostanoids: - prostaglandins, prostacyclins and thromboxanes are each involved in the inflammatory response. In the 1990s,

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researchers discovered that two different COX enzymes existed now known as COX-1 and COX-2. Cyclooxygenase 1 (COX-1) is known to be potent in most tissues. In the gastrointestinal tract COX-1 maintain the normal lining of the stomach. Cyclooxygenese-2 (COX-2) is primarily present at sites of inflammation. Both COX-1 and COX-2 convert arachnidonic acid to prostaglandin, resulting in pain and inflammation. Their other functions make inhibition of COX-1 undesirable while inhibition of COX-2 is considered desirable.

Non-steroidal anti-inflammatory drugs (NSAIDs) commonly prescribed to treat arthritis work by inhibiting prostaglandins. However, they can cause gastrointestinal problems including ulcers. Traditional NSAIDs are considered non-selective because they inhibit both COX-1 and COX-2. Thus COX-2 is a key mediator in gastric wound healing. In contrast, COX-1 has no significant role in healing when COX-2 is impaired, but becomes important when COX-2 is required (Schmassmann, 2005).

Recent research has also highlighted the fact that the protective functions of prostaglandins in the stomach can be carried out by other mediators such as gaseous mediators, nitric oxide and hydrogen sulfide (Ukwa *et al,* 1997; Shigeta *et al,* 1998).

Endogenous PG originating mainly from up regulated COX-2 at the ulcer margin play crucial role in ulcer healing by exogenous PG, PPI, growth factors, gut

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hormones such as gastrin or CCK and melatonin, while COX-1 and COX-2 inhibitors delay ulcer healing by suppressing PG generation, and increasing COX-2 expression in the ulcer area. Thus, COX-2 is a key mediator in gastric wound healing. Many studies have demonstrated the up regulation of COX-2 expression in the ulcerative gastric mucosa, concomitant with an increase in endogenous PG production suggesting that COX-2/PGS play a pivotal role in promoting the healing of gastric ulcers (Araki *et al,* 2002; Halten *et al*, 2001; Mizuno *et al*,1997;). Also Hatazawa *et al*, 2006 reported that endogenous PGE_2 contributes to the healing of induced intestinal ulcer via the activation of EP_4 receptor. The healing promoting action of PGE_2 is associated with the increase of angiogenesis through the up regulation of VEGF expression in the fibroblasts of the gastric ulcer bed or margin via the activation of EP_4 receptors. And since VEGF stimulates cell proliferation, it is possible that PGE_2 via EP_4 receptors promotes not only angiogenesis, but also proliferation and migration of epithelial cells and by so doing contributes to the reconstitution of the ulcerated mucosa in the stomach (Schmassmann, *et al*, 1995). Angiogenesis, the essential component of wound healing is induced by VEGF, which is the fundamental regulator of angiogenesis.

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2.10 IMPORTANCE OF NATURAL PRODUCTS

1. As food supplements e.g. as infant food formula such as dried cow milk.

2. As pharmaceuticals e.g. vitamin D preparation made from fish liver oil and vitamin B6 from wheat germ oil.

3. As industrial intermediates e.g. poppies, *papaver somniferum (papaveraceae)* from which the powerful analgesic morphine is made from. Also the purification of the steroid, hecogenin from the juice of *Agave sisakama (Agavaceae)* and this is used as a synthetic intermediate for cortisone and cortisol production.

4. As templates for new lead discovery such as screening of environmental samples of sediments and surface litter like excreta, decaying plant materials etc. and the micro-organisms contained in them isolated, cultured, and identified for clues of useful secondary metabolites.

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2.11 BETULINIC ACID

The IUPAC name of betulinic acid is (3β)-3-Hydroxy-lup-20(29)-en-28-oic acid (Fig. 2.4). Its molecular formula is $C_{30}H_{48}O_{3}$, with an exact molar mass of 456.3603 and a melting point of 316-318°C (Wikipedia, 2008).

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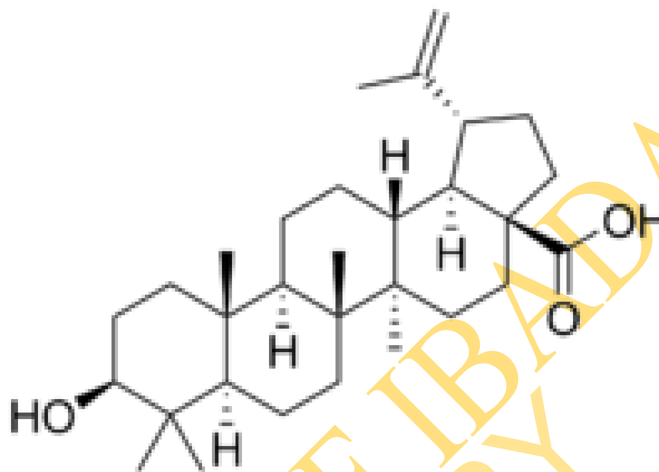

Figure 2.4: Chemical Structure of Betulinic acid (Wikipedia, 2008).

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2.11.1 DISTRIBUTION AND ISOLATION OF BETULINIC ACID

Betulinic acid is a naturally occurring pentacyclic triterpenoid. The lupane-type triterpene betulinic acid is found widely throughout the plant kingdom. Hundreds of published reports have described the occurrence of betulinic acid across a multitude of taxonomically diverse genera. Furthermore, given the widespread occurrence of the structurally related precursor betulin among plants, it is conceivable that the distribution of betulinic acid is even much greater. One of the most widely reported sources of betulinic acid is the birch tree (Betula spp., Betulaceae) where both betulinic acid and betulin can be obtained in substantial quantities. For example, considerable amounts of betulinic acid are available in the outer bark of a variety of tree species, e.g. white-barked birch trees. The reduced congener of betulinic acid, betulin (3β-lup-20(29)-en-3, 28-diol), was one of the first natural products identified and isolated from plants in 1788 (Pezzuto *et al.*, 1999).

A multitude of extraction and isolation schemes have been used for the procurement of betulinic acid and other related triterpenoids. Typically, dry plant material is extracted with $CHCl_3$ (for aglycons) (Kinoshita *et al,* 1998), MeOH (for both aglycons and glycosylated derivatives) (Kinoshita *et al,* 1998; Enwerem *et al,* 2001; Ryu *et al,* 1992) or even H_2O (Macias *et al*, 1995). The plant may be defatted with hexane prior to extraction to remove non-polar materials (Higa *et al,*

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1998; Sun *et al,* 1996). The resultant extracts can be dried and further extracted with other solvents (Enwerem *et al,* 2001; Ryu *et al,* 1992) or directly subjected to column chromatography (Jagadeesh *et al,* 1998; Zhu *et al,* 2000; Ryu *et al,* 2000; Fulda *et al*, 1999; Dunstan *et al*, 1998; Bringmann *et al*, 1997).

2.11.2 SYNTHESIS

Although betulinic acid is widely available from numerous botanical sources, its precursor betulin is available from many plant species in significant yields of >20% (Hayek *et al,* 1989). Furthermore, methods for the multi-ton production of betulin from birch-kraft pulp have been proposed (Hayek *et al,* 1989). Therefore, semi-synthetic methods for the production of betulinic acid from betulin are necessary to provide sufficient quantities of this bioactive triterpene. Two methods have been reported for the scale-up production of betulinic acid from betulin (Kim *et al*, 1997). The first method involves subjecting triterpene betulin to Jones oxidation to give betulonic acid in 75% yield. Reduction of betulonic acid by $NaBH_4$ in THF provides a mixture of 3a- and 3b-hydroxyl products in the ratio 5:95. Crystallization of the product mixture from MeOH affords the 3b-hydroxyl product (betulinic acid) in 75% yield. The remaining product mixture can in turn be re-oxidized and reduced as indicated to give a 71% overall yield of betulinic acid. The second method involves protecting the primary C-28 hydroxyl group in

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betulin and is followed by acetylation of the secondary alcohol at C-3. The protecting group of the C-28 hydroxyl is then removed and the primary alcohol is oxidized to the carboxylic acid. Subsequently, the acetyl moiety is removed yielding betulinic acid in 88% yield. Although this method is efficient, it unfortunately suffers from an increased expenditure of time and materials as compared to the first method.

2.11.3 PHYSICAL PROPERTIES OF BETULINIC ACID

Betulinic acid is a white crystalline solid that exhibits limited solubility in organic alcohols such as methanol and ethanol, trichloromethane, and ether. Betulinic acid has low solubility in water, petroleum ether, dimethylacetamide (DMA), dimethyl Sulfoxide (DMSO), and benzene. However, betulinic acid is highly soluble in pyridine and acetic acid. The crystal structure of betulinic acid has not been reported. However, Bringmann and co-workers (1997) did describe the crystal structure of a betulinic acid benzyl ester derivative.

2.11.4 NMR OF BETULINIC ACID AND DERIVATIVES

NMR methods have indisputably become the single most important spectroscopic technique for the identification and structure elucidation of betulinic acid and its

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analogs. Numerous 1D and 2D NMR methods are now commonly used for the characterization of lupane-type triterpenes.

2.11.5 ABSORPTION, DISTRIBUTION, AND PHARMACOKINETICS

Because of its potential clinical application for the treatment of cancer and HIV infection, studies aimed at determining the fate of betulinic acid in mammals have been initiated. The poor solubility of betulinic acid in aqueous systems generated a great deal of interest in investigating several formulation schemes (Son *et al*, 1998; Rusmawati *et al*, 2001). In one study, a formulation of polyvinylpyrrolidone (PVP)–betulinic acid complex was administered to CD-1 mice as an intraperitoneal dose of 250 or 500 mg/kg (Udeani *et al* 1999). These high dosage levels were selected because they were previously found to be effective in vivo for the treatment of cancer in experimental mice (Pisha *et al,* 1995). Peak serum concentrations of betulinic acid were observed at 0.146 and 0.228 hr for the 250 and 500 mg/kg doses respectively by LC–MS analysis. At the 250 and 500 mg/kg doses, betulinic acid exhibited distribution volumes of 106 and 108 L/kg respectively, and half-lives of 11.5 and 11.8 hr respectively. The distribution of betulinic acid, administered at 500 mg/kg, was found to vary considerably among the various tissues over the course of 24 hour. High concentrations of betulinic acid were found in fat tissues peaking after 24 hour at 2,260 ± 850 mg/g. High

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peak concentrations of betulinic acid were also found in the bladder (3,523 ± 744 mg/g, 8 hr), lymph node (4,218 ± 2,809 mg/g, 4 hr), mammary gland (1,184 ± 904 mg/g, 24 hr), ovary (3,055 ± 1,421 mg/g, 4 hr), spleen (1,287 ± 162 mg/g, 24 hr) and uterus (908 ± 165 mg/g, 24 hr).

Following intraperitoneal injection of a single 500 mg/kg dose of betulinic acid in nude-mice, the concentrations of betulinic acid in the blood, liver, lung, kidney, and tumor determined 24 hour post-injection showed the highest concentration of betulinic acid was found in the tumor (452.2 ± 261.2 mg/g) with virtually none in the blood (1.8 ± 0.5 mg/mL). A significant portion of betulinic acid was also found in the liver (223.9 ± 80.3 mg/g) (Shin *et al,* 1999).

2.11.6 METABOLISM

In an effort to elucidate its metabolic fate in humans, microorganisms were utilized as in vitro model systems to predict and prepare the potential mammalian metabolites of betulinic acid. Microorganisms have long been recognized as appropriate tools for studying mammalian drug metabolism based on the extensive homology between microbial and mammalian metabolic pathways (Clark *et al,* 1991). In a series of pertinent experiments (Kouzi *et al,* 2000; Chatterjee *et al,* 1999; Chatterjee *et al,* 2000), betulinic acid was incubated with resting-cell suspensions of *Cunninghamella spp.* (NRRL 5695), *Cunninghamella elegans*

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(ATCC 9244), *Bacillus megaterium* (ATCC 13368 and 14581), and *Mucor mucedo* (UI-4605). From these studies, a series of oxidized and conjugated metabolites of betulinic acid were obtained. All biotransformation products of betulinic acid were evaluated for antimelanoma activity. In addition to the use of microorganisms as models of its mammalian metabolism, molecular modeling studies have been conducted to predict the sites of metabolism mediated by human cytochrome P450 enzyme systems in betulinic acid. One such system is the human CYP2C9. Human CYP2C9 exhibits selectivity for substrates containing an ionizable carboxylic acid group or an analogous group/isostere, and the usual site of metabolism lies at a fairly well-defined distance from this structural feature. However, there are additional criteria associated with substrate selectivity for CYP2C9 which relate to various other contacts with active site residues, including hydrogen bonding, p–p stacking, and certain hydrophobic interactions. Many of the key regions (substrate recognition sites) governing substrate selectivity within the CYP2 family have been probed using site directed mutagenesis, and a number of particularly important contact points with potential substrates have been identified. Based on the accumulating evidence from mutagenesis and other experimental data, a homology model for human CYP2C9 has been constructed and shown to be consistent with the known substrate selectivity characteristics exhibited by CYP2C9 (Lewis *et al*, 1998).

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Betulinic acid, which contains a free carboxylic acid group, may act as a substrate for human CYP2C9. Hence, a modeling study was undertaken utilizing the constructed CYP2C9 homology model in an attempt to predict the sites of CYP2C9-mediated metabolism in betulinic acid. The betulinic acid molecule was fitted with a previously constructed template of six typical CYP2C9 substrates (tolbutamide, tienilic acid, diclofenac, warfarin, ibuprofen, and naproxen) which had been docked within the CYP2C9 model in an orientation consistent with their known sites of metabolism.

2.11.7 BIOLOGICAL ACTIVITIES OF BETULINIC ACID

Anti-HIV Activity

Pentacyclic triterpenes were capable of inhibiting HIV-1 replication in an in vitro model system. Among these compounds, betulinic acid exhibited an IC_{50} value of 1.4Mm (Fujioka *et al,* 1994). Subsequent derivatization studies were performed on betulinic acid to optimize its anti-HIV activity. These efforts have led to the development of a number of intriguing structure leads with potent anti-HIV activity. The 3-O-(3,3-dimethylsuccinyl) derivatives of betulinic acid and dihydrobetulinic acid (in which the C-20 and C-29 methylene carbons are reduced) exhibited highly potent anti-HIV-1 activity with IC_{50} values of $<3.5 \times 10^{-4}$ mM.

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Antitumour Activity

The antimelanoma activity of betulinic acid was first discovered in 1995 at the University of Illinois at Chicago as part of a National Collaborative Drug Discovery Group supported by the National Cancer Institute. Although, original reports have focused mainly on its melanoma-specific cytotoxicity, there has been a surge in recent evidence detailing a broader anticancer activity profile of betulinic acid. Previous reports indicated that betulinic acid was a melanoma-specific cytotoxic compound. However, more recent evidence indicates that betulinic acid possesses a broader spectrum of activity against other cancer cell types ((Pisha *et al*,1995; Fulda *et al* 1997; Fulda *et al*, 1999; Zuco *et al*, 2002;) Betulinic acid have been shown to act through induction of apoptosis independent of the cell's p53 status; however, another study suggests that betulinic acid may induce p53 up regulation in metastatic melanoma cells (Pisha *et al*,1995; Fulda *et al,* 1997; Rieber *et al,* 1998; Selzer *et al,* 2000; Zuco *et al,* 2002). Incubation of betulinic acid with melanoma cells in vitro resulted in the appearance of characteristic surface blebbing and cytoplasmic shrinking that are indicative of the induction of apoptosis. Further evidence supporting the induction of apoptosis came from investigations showing the formation of characteristic high-molecular-weight DNA fragments and flow cytometry studies (Pisha *et al*, 1995). It was reported that betulinic acid induces apoptosis through the induction of changes in

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mitochondrial membrane potential, production of reactive oxygen species, and permeability transition pore openings (Schmidt *et al* 1997). These processes lead to the release of mitochondrial apogenic factors, activation of caspases, and DNA fragmentation (Fulda *et al,* 1997; Fulda *et al,* 1998; Fulda and Debatin, 2000). In addition, betulinic acid exhibited increased efficacy against melanoma cells grown at a reduced pH (<6.8) (Noda *et al*, 1997; Wachsbeberger *et al,* 2002). This finding was of interest since many tumors produce an acidified extracellular environment that may help in the absorption of betulinic acid (a weak organic acid) by the tumor. It was also demonstrated that heat sensitization resulted in the increased susceptibility of tumor cells to betulinic acid possibly because of altered (lowered) intracellular pH (Wachsbeberger *et al,* 2002). A previous investigation had shown that betulinic acid inhibited the activity of aminopeptidase N in vitro, an endogenous angiogenic factor, but failed to inhibit the enzyme in vivo (Melzig and Bormann, 1998; Kwon e*t al,* 2002). Betulinic acid does, however, inhibit mitochondrial function in endothelial cells (Kwon e*t al* (2002) Betulinic acid has also been shown to protect congenital melanocyte naevi cells from UV-C-induced DNA strand breakage independent of p53 and p21. It was speculated that betulinic acid may be acting through an antioxidant mechanism (Salti *et al*, 2001). However, betulinic acid was shown to potentiate the activity of bleomycin, a mediator of DNA strand breakage, through inhibition of DNA polymerase in P-388D1 cells

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(Ma *et aI,* 1999). Additionally, betulinic acid exhibits weak inhibitory effects against topoisomerase I and IIa, but does not stabilize the topoisomerase IIa–DNA complex (Syrovets et *al,* 2000). Betulinic acid is active in vivo against TPA-induced tumors and ovarian and melanoma xenographs in mice and rats (Yasukawa *et al,* 1991; Yasukawa *et al,* 1995; Pisha *et al,* 1995; Zuco *et al,* 2002). Remarkably, betulinic acid exhibited no toxic effects in mice even at a concentration of 500 mg/kg (Pisha *et al* 1995). However, doses of betulinic acid as low as 5 mg/kg were determined to significantly impede tumor development (Pisha *et al,* 1995). Even more striking are the results of a study in which betulinic acid was withheld from mice infected with melanoma cells for 41 days and then administered as a six-dose regimen (50 mg/kg each) every third day. At the time of autopsy (71 days), the mice exhibited greater than 80% regression in tumor size (Pisha *et al,* 1995).

Antitumour mode of action

Regarding the mode of action of betulinic acid, little is known about its antiproliferative and apoptosis-inducing mechanisms. In neuroectodermal tumor cells, betulinic acid–induced apoptosis is accompanied by caspase activation, mitochondrial membrane alterations and DNA fragmentation (Fulda *et al,* 1997; Thurnher *et al,* 2003). Caspases are produced as inactive proenzymes, which are

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proteolytically processed to their active forms. These proteases can cooperate in proteolytic cascades, in which caspases activate themselves and each other. The initiation of the caspases cascade may lead to the activation of endonucleases like caspase-activated DNAase (CAD). After activation CAD contributes to DNA degradation (Thurnher *et al*, 2003). Betulinic acid induces apoptosis by direct effects on mitochondria, leading to cytochrome-c release, which in turn regulates the "downstream" caspase activation (Thurnher *et al,* 2003). Betulinic acid bypasses resistance to CD95 and doxorubicin-mediated apoptosis, due to different molecular mechanism of betulinic acid-induced apoptosis.

Controversial is a role of p53 in betulinic acid-induced apoptosis. It has been suggested that p53-independent mechanism of the apoptosis is increased after treatment with doxorubicin. (Fulda *et al*, 1997). The suggestion is supported by study of Raisova *et al*. (2001). On the other hand, it has been suggested that betulinic acid exerts its inhibitory effect on human metastatic melanoma partly by increasing p53 (Rieber and Strasberg, 1998).

The study also demonstrated preferential apoptotic effect of betulinic acid on C8161 metastatic melanoma cells, with greater DNA fragmentation and growth arrest and earlier loss of viability than their non-metastatic C8161/neo 6.3 counterpart (Rieber and Strasberg, 1998). Comparing the betulinic acid with other treatment modes, Zuco *et al*, 2002, demonstrated that it was more than 10 times

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less potent than doxorubicin (IC50 4.5 µg/ml Vs IC50 0.21-034 µg/ml in doxorubicin) and showed an in vitro antiproliferative activity against melanoma and non-melanoma cell lines, including those resistant to doxorubicin. On the human normal dermatoblast cell line betulinic acid was 2-5 times less toxic than doxorubicin (Zuco *et al*, 2002), The ability of betulinic acid to induce two different effects (cytotoxic and cytostatic) on two clones derived from the same human melanoma metastasis suggests that the development of clones resistant to this agent will be more unlikely, than that to conventional cytotoxic drugs. Moreover in spite of the lower potency compared with doxorubicin betulinic acid seems to be selective for tumor cells with minimal toxicity against normal cells (Zuco *et al,* 2002). The effect of betulinic acid on melanoma cell lines is stronger than its growth-inhibitory effect on primary melanocytes (Selzer *et al*, 2000). Study of combination of betulinic acid with γ-irradiation showed clearly additive effects, and indicates that they differ in their mode of action (Selzer *et al,* 2000)

Anti-Inflammatory Properties

A large number of pentacyclic triterpenes have been investigated for anti-inflammatory properties.(Safayhi and Sailer, 1997). Among these are the lupane-type triterpenes betulinic acid and betulin. Both of these compounds have been tested in a number of in vitro and in vivo model systems. Combined, studies

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suggest that both betulinic acid and betulin possess moderate anti-inflammatory properties at relatively high concentrations. Studies have indicated that the anti-inflammatory effects of betulinic acid and betulin are attributed to the inhibition of non-neurogenic pathways (Huguet *et al,* 2000). It has been suggested that the activity of betulinic acid is in part the result of interaction with glucocorticoid receptors; however, the de novo synthesis of endogenous anti-inflammatory proteins may play a role as well (Recio *et al,* 1995; Mukherjee *et al,* 1997).

Other Biological Activities

Betulinic acid has been tested for a variety of other biological activities. Several studies have examined the potential antimicrobial effect of betulinic acid (Nick *et al,* 1995; Jeong *et al,* 1999; Fulda *et al,* 1999; Wachter *et al*, 1999). Betulinic acid has been shown to be inactive in vitro against *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* (Nick *et al,* 1995; Fulda *et al,* 1999; Schuhly *et al,* 1999). In one instance, betulinic acid exhibited a MIC value of 32 mM against *Mycobacterium tuberculosis* (Wachter *et al,* 1999). In other experiments, the MIC values of several phenylester derivatives of betulinic acid ranged from 8 to 16 mg/mL against *Staphylococcus epidermidis* (Schuhly *et al,* 1999). Betulinic acid has also been tested against the essential fungal enzyme *chitin synthase II* where it exhibited IC50 value of 98.7mg/Ml

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(Jeong *et al,* 1999). Other studies showed betulinic acid to be non-toxic to *Penicillium citrinum* (Higa *et al,* 1998). Collectively, these reports indicate that betulinic acid is not a promising antimicrobial agent.

Betulinic acid has also been evaluated for antimalarial activity against *Plasmodium falciparum*. Bringmann *et al* (1997) showed that betulinic acid exhibited an in vitro IC_{50} value of 10.46 mg/mL against *P. falciparum*. The in vitro antimalarial activity of betulinic acid was also reported against two strains of *P. falciparum*- K_1 and *T9-96*, with IC_{50} values of 19.6 and 25.9 mg/mL, respectively (Steele *et al,* 1999). However, in vivo experiments in mice infected with *Plasmodium berghei* showed that betulinic acid had no activity at concentrations up to 250 mg/kg/day (Steele *et al,* 1999). Recently, several C-3 and C-28 betulinic acid derivatives have been shown to exhibit improved in vitro activity against *P. falciparum* (Pathak *et al,* 2002). Several terpenes including betulin and betulinic acid were examined for their spasmogenic activity. The two compounds were tested in vitro against isolated rat fundus at 1 x 10^{-4} M. Both betulinic acid and betulin demonstrated partial agonistic effects relative to serotonin and produced 46.5 and 60.5% contractile response respectively (Bejar *et al*, 1995).

Betulinic acid has been reported to exhibit a variety of other biological effects. Studies revealed that betulinic acid possessed antinociceptive, antihelmintic and anti-HSV-1 activities (Ryu *et al,*1993; Kinoshita *et al,*1998 ; Enwerem *et al,*

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2001). In addition, betulinic acid has been shown to exert inhibitory activity against cyclic AMP-dependent protein kinase, sulfonylureas, stromelysin and collagenase (Wang and Polya ,1996; Zhu *et al,* 1996; Sun *et al* 1996). Betulinic acid and derivatives do not exhibit tobacco larvae antifeedant spermicidal, or icthyotoxic activities (Purohit *et al,* 1993; Higa *et al,*1998; Jagadeesh *et al,* 1998).

2.12 TOXICITY OF BETULINIC ACID

Studies have shown that betulinic acid exhibits greatly reduced in vitro cytotoxicity against normal dermal fibroblast and peripheral blood lymphocytes (Zuco *et al,* 2002). However, intraperitoneal injections of betulinic acid administered to mice pre-infected with Plasmodium berghei, at a dose of 250 mg/kg/ day for 4 days, resulted in the death of one of the experimental mice (Steele *et al,* 1999). In contrast, a Hippocratic screen of betulinic acid and some of its derivatives, administered intraperitoneally to rats at doses of 200 and 400 mg/kg, provided no evidence of toxicity (Sandberg *et al,* 1987). Likewise, Pisha and co-workers (1995) reported no toxicity associated with the intraperitoneal administration of six doses (500 mg/kg each) of betulinic acid to mice and further tests using a similar treatment regimen of six doses (250 mg/kg each) of betulinic acid were also non-toxic. In vitro studies utilizing Madin Darby kidney cells did reveal that betulinic acid is responsible for a significant increase in the concentration of intracellular-

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free calcium; however, the increase in free calcium levels was associated with only a slight decrease in cell viability (Chou *et al.* 2000). Based on these findings, it appears that betulinic acid possesses very little broad cytotoxicity, if any, at relatively high therapeutic doses.

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Feeds, Sensitive weighing balance, dissecting set, animal cages, laboratory glassware, cotton wool, plain specimen bottles, Petri dishes, syringes, albino rats, spectrophotometer (Perkin Elmer UV visible spectrophotometer Lambda 3B), operating table, beakers, conical flasks, water bath, glass slides and cover slips, optical microscope, micrometer screw gauge, homogenizer, modified Langerdoff apparatus.

3.2 CHEMICALS

Betulinic acid (BDH Aldrich Sigma), sodium chloride, sodium hydroxide, eosin, haematoxylin, Dimethyl sulphuroxide (DMSO), Sodium acetate (M&B, England), Analar Sucrose (BDH Chemical Ltd, Poole England), Magnesium chloride, Alcian blue, Formalin, Diethyl ether, Trichloroacetic acid, indomethacin (Sisbui Xiedang, China).

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3.3 SOLUTIONS

Normal saline, magnesium chloride solution, sucrose solution, sodium acetate, alcian blue solution, hydrochloric acid, 0.0025N sodium hydroxide, diethyl ether, Thiobarbituric acid (0.75%), Trichloroacetic acid (30%), 10% formalin, Betulinic acid (BDH Aldrich Sigma) dissolved in Dimethyl sulphuroxide (DMSO), Histamine acid phosphate, 1% phenolphthalein solution, 5mg/ml indomethacin.

3.3.1 PREPARATION OF STOCK SOLUTIONS AND REAGENTS

(a) Normal saline: The solution was prepared by dissolving 0.9g sodium chloride in 100ml of distilled water.

(b) 0.1M Alcian blue solution: The solution was prepared by first dissolving 0.1g of alcian blue in 100ml of distilled water, and the resultant solution dissolved in 0.16M sucrose solution. This is then buffered with 0.05M sodium acetate and then adjusted to a pH of 5.8 using hydrochloric acid.

(c) 0.16M Sucrose solution: The solution was prepared by dissolving 5.47g of sucrose in 100ml of distilled water.

(d) 0.25M Sucrose solution: This was prepared by dissolving 8.55g of sucrose in 100ml of distilled water.

(e) 0.05M Sodium acetate solution: This was prepared by dissolving 0.4g of sodium acetate in 100ml of distilled water,

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(f) 0.5M Magnesium chloride: This was prepared by dissolving 4.75g of magnesium chloride in 100ml of distilled water.

(g) Betulinic acid solution: This was prepared by dissolving 100mg of Betulinic acid in 5ml of DMSO and then making it up with distilled water.

(h) 5mg/ml indomethacin: This was prepared by dissolving 25mg of the drug in 5ml of distilled water with 2% sodium carbonate.

(i) 0.0025N sodium hydroxide: The stock solution was prepared by dissolving 4g of sodium hydroxide (NaOH) pellets in 100ml of distilled water. From this stock solution, 1ml was taken and made up to 400ml with distilled water to get the required concentration of 1/400M (0.0025N).

(j) 1% phenolphthalein solution: Obtained by dissolving 1mg of phenolphthalein salt in 50ml of absolute alcohol. After the dissolving of the salt, 50ml of distilled water was added to the solution.

3.4 EXPERIMENTAL DESIGN

This experiment tends to carry out six studies, they are:

1. To determine the effect of Betulinic Acid (BA) on indomethacin -induced gastric ulceration.
2. To determine the effect of Betulinic acid on gastric mucus secretion.
3. To determine the effect of Betulinic acid on gastric mucus cell count.

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4. To determine the effect of Betulinic acid on basal and histamine-stimulated gastric acid secretion.

5. To determine the anti-oxidant effect of Betulinic acid through the determination of Malonialdehyde (MDA) concentration. .

6. To determine the histological changes due to the effect of betulinic acid on gastric mucosal cells.

3.5 CHOICE OF ANIMALS AND EXPERIMENTAL PROCEDURE

A total of one hundred and ninety-two (192) adult male albino rats of Wistar strains with average weight of between 230 and 250 grams. The choice of male rats was to obtain a fairly constant physiological condition, as gastric acid secretion does vary with oestrous cycle in female rats (Amure and Omole, 1970). The animals were purchased from the central animal house, College of Medicine, University of Ibadan, Nigeria. They were housed in a clean, well-ventilated room and maintained under standard condition (12 hours light and 12 hours darkness). The animals were allowed to acclimatize before the study commences. They were fed with commercial rat chow obtained from Ladokun Livestock Feed Limited, Ibadan Oyo State Nigeria and water was provided *ad libitum*.

The one hundred and ninety-two (192) rats were randomly assigned into six different experimental groups (Group 1-6) of thirty two animals per group. Each

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group was used for a study and was further divided into four sub-groups of eight animals each. For each study, Sub-group 1 served as the control and was treated orally with DMSO in normal saline, while Sub-group 2, Sub-group 3 and Sub-group 4 were each treated with Betulinic Acid at doses/ body weight of 0.5 mg/kg, 1.5 mg/kg and 3.0 mg/kg orally respectively for seven days.

3.5.1 EFFECT OF BETULINIC ACID ON INDOMETHACIN INDUCED GASTRIC ULCERATION

A total of thirty-two (32) rats were used for this study divided into four groups, each with eight rats. Group 1 was the control and was treated with DMSO in normal saline. Group 2, Group 3 and Group 4 were treated with BA doses of 0.5mg/kg, 1.5 mg/kg, 3.0 mg/kg orally respectively (Durst *et al*, 2002).The animals were fasted for 24 hours only but allowed free access to water. The method of indomethacin induced gastric ulceration adopted was that described in previous works (Njar *et al*, 1995; Oluwole *et al*, 2008). One hour after the administration of betulinic acid and DMSO in normal saline, indomethacin at 40mg/kg BW (Merck, Sharp & Dohme, Canada) was administered subcutaneously to all the animals in all the groups. After 4 hours, the animals were sacrificed by cervical dislocation. Their stomachs were removed, opened by cutting along the whole length of the greater

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curvature, turned inside out and then pinned to a cork mat. This was moistened with normal saline to prevent autolysis. The method used for assessment of the degree of gastric ulceration was that of Alphin and Ward (1967) as modified by Elegbe and Bamgbose (1976). Macroscopic examinations of the washed stomachs were carried out with a magnifying hand lens. The ulcer scoring system used was as follows:

0 = Normal Stomach

0.5 = Punctuate haemorrhage or pin point ulcers

1.0 = Two or more haemorrhagic ulcers

2.0 = Ulcers greater than 3mm in diameter

$$\text{Mean Ulcer Score} = \frac{\text{Total Ulcer Score}}{n}$$

Where n = number of rats

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3.5.2 EFFECT OF BETULINIC ACID ON GASTRIC MUCUS SECRETION

A total of thirty-two (32) rats used for this study divided into four groups, each with eight rats and treated orally on a daily basis for 7 days. Group 1 was the control and was treated with DMSO in normal saline. Group 2, Group 3 and Group 4 were treated with BA doses of 0.5mg/kg, 1.5 mg/kg, 3.0 mg/kg orally respectively (Durst *et al*, 2002).

The rats were sacrificed by cervical dislocation and their stomachs were removed and weighed. The glandular portion of each stomach was opened along the lesser curvature. Procedures for measurement were that described by Corney et al (1974). The absorbance of each solution was used to calculate the various concentrations of dye and the weight of dye (expressed in mg) was deduced, using a standard curve. The weight of the dye was expressed over the weight of the stomach to give the weight of the mucus secreted.

Thus, gastric mucus secretion (mg/g tissue) = $\frac{\text{Weight of dye (mg)}}{\text{Weight of stomach (g)}}$

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3.5.3 EFFECT OF BETULINIC ACID ON GASTRIC MUCUS CELL COUNT

A total of thirty-two (32) rats were used for this study. They were divided into four groups, each with eight rats and treated orally on a daily basis for 7 days. Group 1 was the control and was treated with DMSO in normal saline. Group 2, Group 3 and Group 4 were treated with BA doses of 0.5mg/kg, 1.5 mg/kg, 3.0 mg/kg orally respectively (Durst *et al*, 2002).

The rats were sacrificed by cervical dislocation, the stomachs removed and weighed. The glandular portion of the each stomach was opened along the lesser curvature.

Gastric mucus cell count was done by counting the number of gastric mucus cells that stain with Haematoxylin and Eosin. These are indicated as blue patches. The stained slide of the stomach mucosa of each rat was viewed under the microscope. The gastric mucus cells were counted using calibrated microscope in five randomly selected area of the gastric mucosal tissue. Five cubic boxes each with an area of $1mm^2$ were assessed. This method is an improvement over the earlier described approach for counting by Li *et al* (2002).

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3.5.4 EFFECT OF BETULINIC ACID ON BASAL AND HISTAMINE-STIMULATED GASTRIC ACID SECRETION.

The basal acid secretion and the maximal acid secretion were measured. A total of thirty-two (32) rats were used for this study. They were divided into four groups, each with eight rats and treated orally on a daily basis for 7 days. Then the animals were fasted for 24 hours but allowed free access to water. Group 1 was the control and was treated with DMSO in normal saline. Group 2, Group 3 and Group 4 were treated with BA doses of 0.5mg/kg, 1.5 mg/kg, 3.0 mg/kg orally respectively (Durst *et al*, 2002).

BASAL SECRETION

Each rat was anaesthetized with urethane according to their body weights (0.6 ml per 100g BW). The animals were then tied to the dissecting board and dissected. The neck of the animal was dissected at the midline of the ventral surface, the connective tissue removed to expose the trachea. A small cut was made in the upper part of the trachea and cannulated. This was to ensure that the airway was clear. Mucus was removed from the airway using a moist cotton wool. A size 3-cannula from the modified Langerdoff apparatus was passed into the esophagus, care being taken not to puncture the esophageal wall. The cannula was push until it

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could be felt in the cardiac region of the stomach. A ligature was tied around the esophagus to secure the cannula. The fur on the lower abdominal portion was shaved and a midline incision was made through the skin so as to bring out the stomach. A small cut was made an inch distal to the pylori-duodenal junction and through it the stomach was washed by the normal saline fluid until clear effluent was observed. The duodenum was cannulated and tied. The stomach was put back into the peritoneum and the cut surface closed and covered with moist cotton wool. The end of the cannula was then put into a beaker to collect the effluent.

The femoral vein was exposed by dissection, cut made in the upper thigh with the femoral sheath containing the femoral vein, artery, and nerve exposed. The vein was isolated and ligated in two places, the toe-ward, and the body-ward directions. Blood flow through the vein was first occluded by holding the vein with a bulldog clip in the body-ward direction. The vein was made to distend fairly by pushing blood towards the clip. A femoral cannula was inserted in the femoral vein in the toe-ward direction and tied.

After cannulating the femoral vein, the rate of flow of the perfusing fluid from the Langerdoff apparatus was adjusted. The rate of perfusion was regulated such that 10ml of gastric contents was collected from the stomach cannula at 10 minutes interval. This technique is known as the continuous perfusion technique method of

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Ghosh and Schild (1988). The collected effluent was titrated with 0.0025N NaOH after adding two drops of phenolphthalein to it.

MAXIMUM SECRETION

A dose of 0.1mg/g body weight histamine acid phosphate was injected intravenously (i.v.) through the femoral vein into the rats. Then 10ml of gastric contents was collected after 15 minutes, after which the timing for 10ml collection was made every 10 minutes for about an hour.

PREPARATION AND THE DOSAGE VALUE OF HISTAMINE

A histamine stock solution of 20mg/ml concentration was gotten from the laboratory of the Department of Physiology, University of Ibadan. Since this stock solution was quite of a higher concentration, it was then diluted to fit.

The dosage value that was to be administered is 50mg/kg (Thompson *et al*, 1967).

Calculating,

1ml of the stock solution was gotten

Then, it was mixed with 9mls of distilled water.

Therefore, concentration becomes 20mg/10mls = 2mg/ml

Dosage to be administered = 50mg/kg = 5mg/100g.

The volume of the above solution to be given $= \frac{\text{Dosage value}}{\text{Concentration}} = \frac{5\text{mg}/100\text{g}}{2\text{mg/ml}}$

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= 2.5mls/100g.

Therefore, 2.5mls of the above solution of histamine with a concentration of 2mg/ml was administered to each rat of 100g body weight.

EXAMINATION OF SAMPLES

The total acidity of the gastric contents was determined by using the titrating method with an initial drop of 1% Phenolphthalein. This was titrated with 0.0025N NaOH from a burette. The end point was determined when the solution turns pink.

PREPARATION OF URETHANE

Urethane granules were obtained from the department of Physiology. 25 g was weighed out and dissolved in 100 ml of distilled water to give a concentration of 25% w/v urethane. Dose of drug administered = 0.6 ml/100 g/BW.

Therefore, 0.6 ml of 0.25g/ml of urethane was administered per100g weight of an animal.

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VOLUMETRIC ANALYSIS

At the end of the titration process, the following calculations were carried out:

$$M_A V_A = M_B V_B \quad \text{...} (1)$$

Where M_A = Molarity of acid

V_A = Volume of acid

M_B = Molarity of base

V_B = Volume of base

It follows that $M_A = \frac{M_B V_B}{V_A}$ .... (2)

But $M_A = \frac{\text{Concentration, C}}{\text{Gram Equivalent weight, G}}$

$C = M_A \times G$ .... (3)

Substituting for M_A in equation 2 into 3

$$C = \frac{M_B V_B}{V_A} \times G \text{ g/litre}$$

Therefore C in mg/litre will be

$C = \frac{M_B V_B}{V_A} \times G \times 1000$ .... (4)

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But Meq/litre = $\frac{\text{Conc value in mg/1000 ml x 10}}{\text{Gram equivalent weight G}}$ ……………………... (5)

Substituting for Conc. Value in 10mg/1000 ml and substituting equation 4 into 5,

$$\text{Meq/litre} = \frac{M_B V_B \times G \times 1000 \times 10}{V_A \times 10 \times G}$$

$$= \frac{M_B V_B \times 1000}{V_A} \quad \text{……………………………………… (6)}$$

But since M/400 NaOH was used = M_B = 1/400 M

Substituting for M_B

$$\text{Meq/litre} = \frac{\frac{V_B}{400} \times 1000}{V_A}$$

$$= \frac{5V_B}{2V_A}$$

But 10ml of acid was used for each titration i.e V_A = 10ml

Therefore M eq/litre = $\frac{5V_B}{2 \times 10}$ = 0.25 V_B

3.5.5 ANTI-OXIDANT EFFECT OF BETULINIC ACID

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MDA level was assessed using the method described by Gutteridge and Wilkins (1982) by measuring Thiobarbituric acid reactive substances (TBARS) produced. A total of thirty-two (32) rats were used for this study. They were divided into four groups, each with eight rats and treated orally on a daily basis for 7 days. Group 1 was the control and was treated with DMSO in normal saline. Group 2, Group 3 and Group 4 were treated with BA doses of 0.5mg/kg, 1.5 mg/kg, 3.0 mg/kg orally respectively (Durst *et al*, 2002).

PRINCIPLE

This method is based on the reaction between 2-thiobarbituric acid (TBA) and malonialdehyde (MDA) which is an end- product of lipid peroxidation. On heating in acidic solution, a pink coloured complex was produced that absorbs maximally at 532 nm on the spectrophotometer. 0.1ml of the test sample was mixed with 0.5ml of 10% TCA and 0.5ml of 75% TBA was then added. The mixture was placed in water bath at 80°C for 45 minutes. The absorbance of the resulting pink colour solution was measured against a reference blank of distilled water at 532nm. The test sample was calibrated using the MDA as standard and the result was expressed as the amount of free MDA produced or MDA quantified by using the molar extinction coefficient, C of 1.56 x $10^{5}M^{-1}cm^{-1}$ according to the expression of Adam Vizi and Seregi (1982).

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MDA (units/g tissue) = $\frac{\text{Absorbance of sample}}{\text{Molar extinction coefficient}}$

3.5.6: EFFECT OF BETULINIC ACID ON THE HISTOLOGICAL CHANGES OF THE GASTRIC MUCOSAL.

A total of thirty-two (32) rats were used for this study. They were divided into four groups of eight rats and treated daily for 7 days orally. Group 1 was the control and was treated with DMSO in normal saline. Group 2, Group 3 and Group 4 were treated with BA doses of 0.5mg/kg, 1.5 mg/kg, 3.0 mg/kg orally respectively (Durst *et al*, 2002).

After the 7 days treatment period, the rats were sacrificed by cervical dislocation, the stomach removed and weighed. The glandular portion of the stomachs were opened along the lesser curvature, rinsed, and placed into plain sample bottle containing 10% formalin. These were used to prepare histological slides using haematoxylin and eosin.

3.6 Statistical Analysis

Data were expressed as mean ± SEM. Student's t-test and one way analysis of variance (ANOVA) were used to analyze the data. P- value less than 0.05 were considered statistically significant.

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CHAPTER FOUR

4.0 Results

4.1 Effect of Betulinic Acid (BA) on Indomethacin Induced Gastric Ulceration

The mean ulcer score decreases with increasing doses of BA when compared with the control. Each administered dose of 1.5 mg/kg BA (0.8± 0.10) and 3.0 mg/kg BA (0.3± 0.09) compared to the control rats (7.0± 0.27) showed significant reduction in mean ulcer score ($p < 0.05$). However, the difference in mean ulcer score for 0.5 mg/kg dose of BA (6.3 ± 0.42) and the control (7.0± 0.27) was not significant ($p > 0.05$). Also it was noticed that, as the dose of betulinic acid increases in the treated animals, percentage inhibition tends toward 100% (Table 4.1). This shows a lower incidence of ulceration. This inhibition of gastric ulceration by betulinic acid is therefore dose dependent

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Table 4. 1: The Effect of Betulinic Acid (BA) on Indomethacin Induced Gastric Ulceration

Treatment	Mean Ulcer score [a]	Inhibition of ulceration (%) [b] = $\frac{(\text{control} - \text{Trt})}{\text{control}} \times 100$
Normal Saline + DMSO (control)	7.0 ± 0.27	-
BA (0.5mgkg^{-1})	6.3 ± 0.42	10.7
BA (1.5mgkg^{-1})	0.8 ± 0.10 *	89.3
BA (3.0mgkg^{-1})	0.3 ± 0.09 *	96.4

Trt = Treated,

[a.] Values are Mean ± SEM, for 8 animals per group. *$p< 0.05$ significantly lower compared with control.

[b] Percentage inhibition as described by Raji et al, 2000.

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4.2: Effect of Betulinic Acid on Gastric Mucus Secretion

As shown in fig. 4.1, there was significant increase in gastric mucus secretion with doses 1.5 mg/kg BA (4.9± 0.22) and 3.0 mg/kg BA (5.2± 0.09) treated rats compared to the control rats ($p<0.05$). This increase is dose dependent. However increase in gastric mucus secretion at a dose of 0.5 mg/kg BA (4.6 ± 0.16) as compared to the control (4.4 ± 0.20) was not significant ($p<0.05$).

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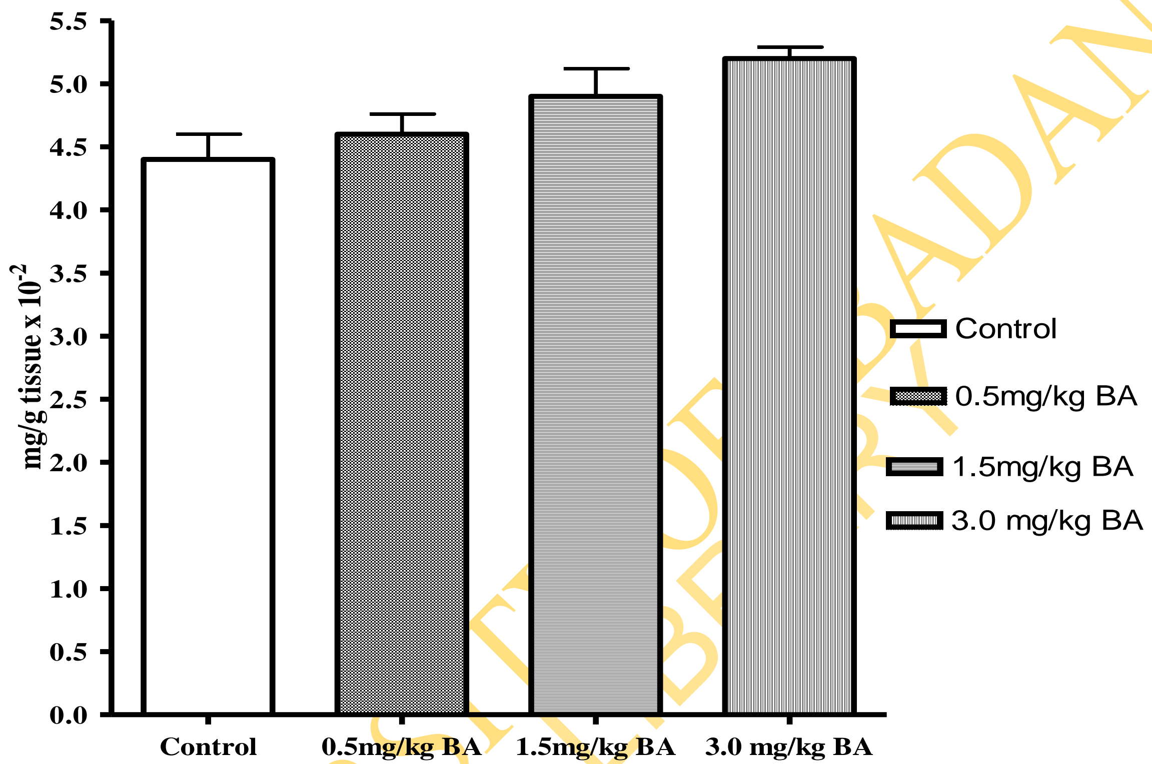

*p<0.05 significant compared to the control

Figure 4.1: Effect of Betulinic Acid on Gastric Mucus Secretion

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4.2: Effect of Betulinic Acid on Gastric Mucus cells count (mm^2)

The effect of BA on gastric mucus cells count showed graded increase with the increasing doses (Fig. 4.2). There is a significant increase in the mucus cells count with dose 0.5 mg/kg BA treated rats (44.4 ± 0.84) compared to the control rats (41.4 ± 0.7) ($p < 0.05$). Similarly, the doses of 1.5 mg/kg BA (45.9 ± 0.79) and 3.0 mg/kg BA (54.1 ± 1.07) produced increases in gastric mucus cells count, that are significantly different compared to the control rats group (41.4± 0.71) ($p < 0.05$).

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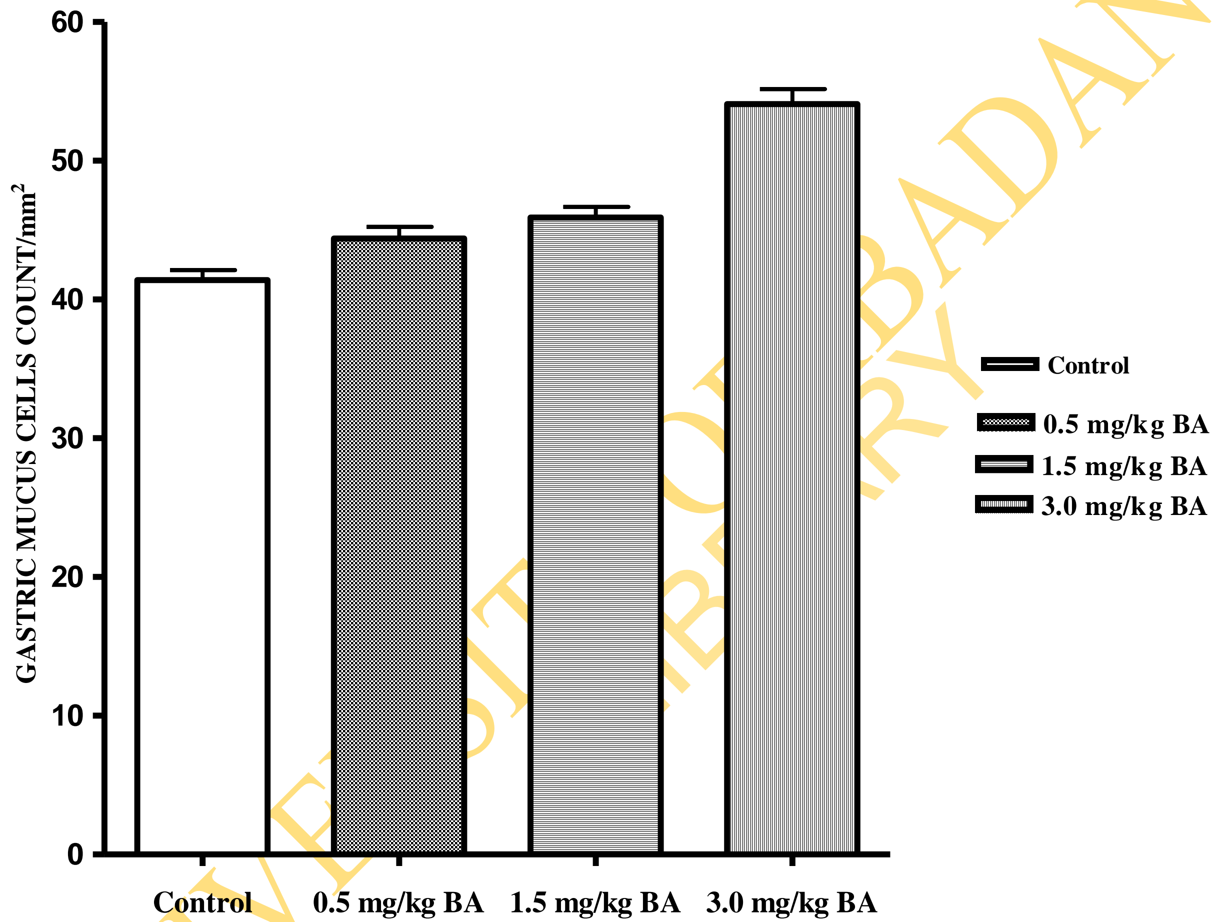

*$p<0.05$ significant compared to the control

Figure 4.2: Effect of Betulinic Acid on Gastric Mucus cells count (mm^2)

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4.3: Effect of Betulinic acid on Gastric acid secretion

Figure 4.3 represents basal gastric secretion in all the rats groups and after the administration of histamine. In BA treated rats groups, there were significant reductions in gastric acid secretion after histamine administration when compared to the control ($p < 0.05$). With administration of 0.5 mg/kg BA and 1.5 mg/kg BA, significant reduction in gastric acid secretion was noticed compared to the control group ($p < 0.05$). So also, gastric acid secretion significantly reduced with the dose of 3.0 mg/kg BA compared to the control ($p < 0.05$). Ten minutes post histamine administration showed significant reduction in gastric secretion in each of the BA treated rats groups as compared to the control ($p < 0.05$).

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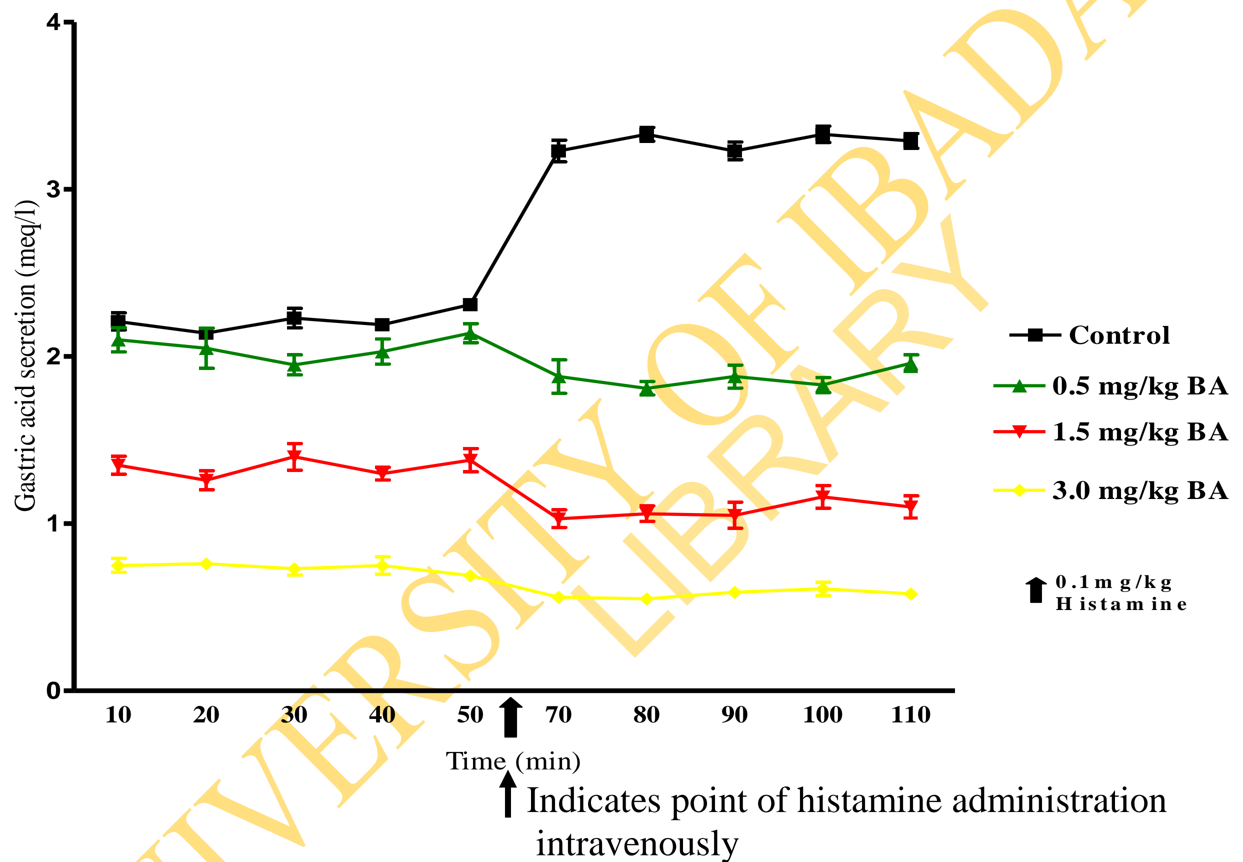

Figure 4.3: Effect of Betulinic acid on Gastric acid secretion

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4.4 Effect of Betulinic Acid on Malonialdehyde (MDA) Concentration

Fig. 4.4 shows the effect of BA on MDA concentration. There was a significant decrease in MDA with 0.5 mg/kg BA treated rats (1.86± 0.076) as against the control rats (2.60± 0.110) ($P < 0.05$). So also the MDA concentration in each of the 1.5 mg/kg BA treated rats (1.83± 0.069) and the 3.0 mg/kg BA treated rats (1.10± 0.056) showed significant decrease compared to the control (2.60± 0.110)($p < 0.05$). This decrease exhibits dose dependence.

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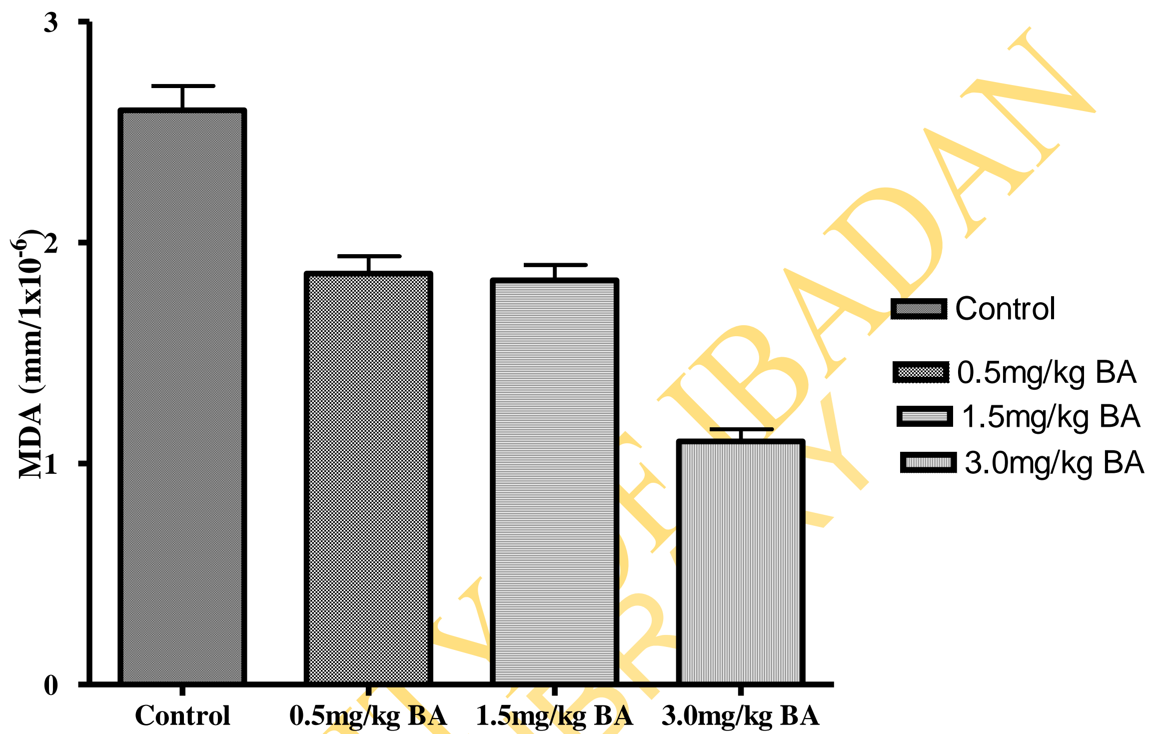

*p<0.05 significant compared to the control

Figure 4.4: Effect of Betulinic Acid on Malonialdehyde (MDA) Concentration

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4.5 Effect of Betulinic Acid on the Histology of the Rat Stomach

The mucosal appears normal with an intact layer of surface mucus cells lining the gastric pits. The arrow points to a mucus cell appearing as dark blue spot to H&E stain and population of mucus cells not numerous. The white spaces are the mucus pits.

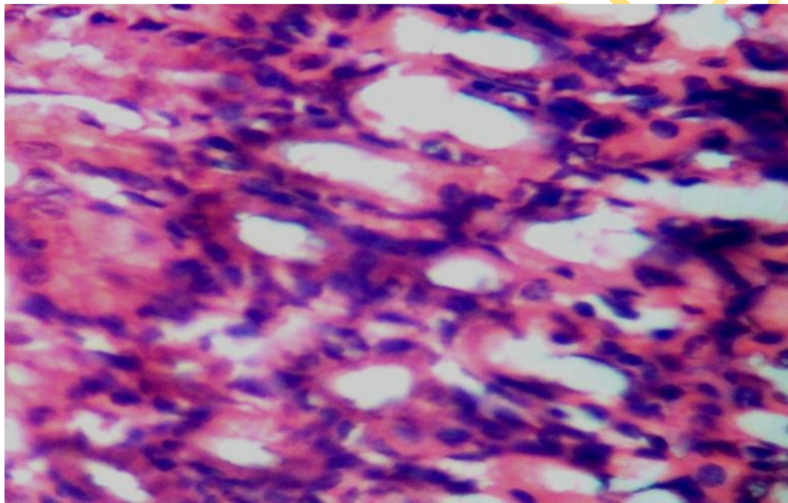

Plate 1. A photomicrograph of a normal (Control) rat stomach (x400).

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The arrow points to a mucus cell. The dark blue spots are the nuclei of the mucous cells while the whitish appearance are the mucus within the cells, thus the mucus cells are fairly numerous, showing increase in the number of mucus cells, so also are the mucus spaces.

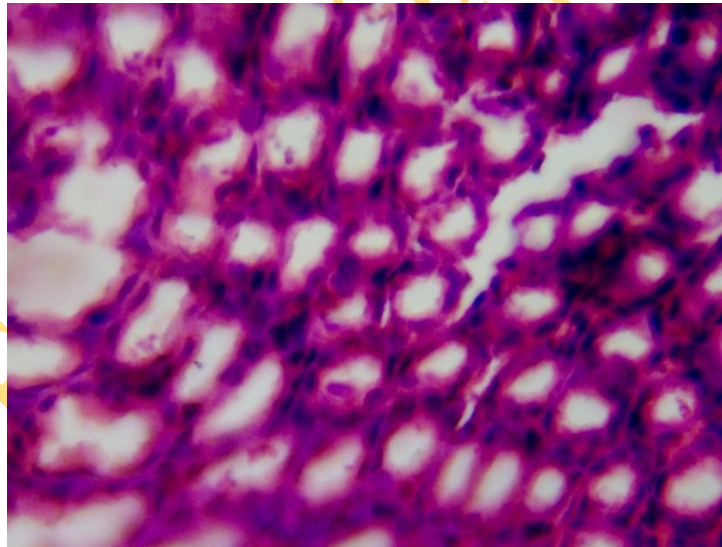

Plate 2. A photomicrograph of the stomach of a rat treated with 0.5mg/kg BA (x400).

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The surface mucus cells appear more prominent. The dark spots (mucus cells) are significantly more numerous than in the control and 0.5mg/kg BA pretreated rats group. Thus there seems to be more functional mucus cells compared to the control group. The arrow points to a mucus cell.

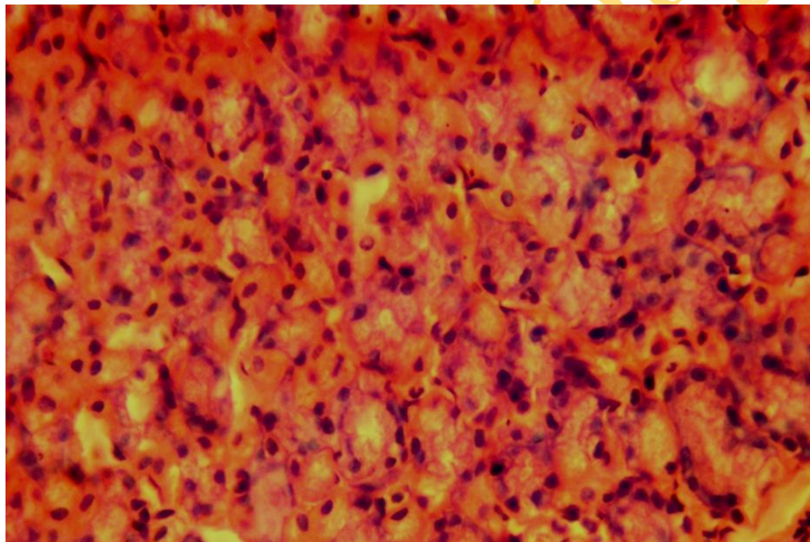

Plate 3: A photomicrograph of the stomach of a rat treated with 1.5mg/kg BA (x400).

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More surface mucus cells are more numerous and are found disintegrating into spaces of the gastric pits compared to the control group. There seems to be more functional mucus cells compared to the control group. The arrow points to a mucus cell.

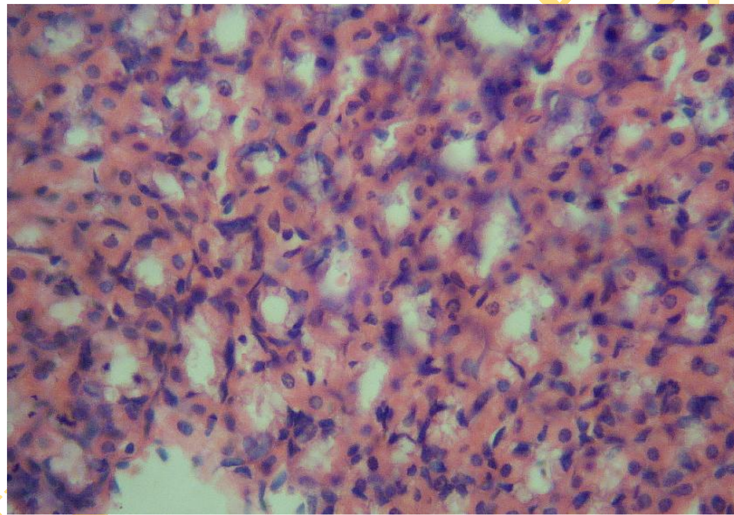

Plate 4: A photomicrograph of the stomach of a rat treated with 3.0 mg/kg BA (x400).

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CHAPTER FIVE

5.0 DISCUSSION

The mechanism of anti-gastric ulcer effect of betulinic acid (BA) was investigated in this study. The role of gastric acid in the etiology of ulcer has been well studied (Baron, 1963). Also histamine formation and release in the gastric mucosa have been implicated in the pathogenesis of gastric ulcers; antihistamine agents may be useful in the prevention of such lesion (Parmar and Ghosh, 1981). The gastric acid stimulatory action of histamine is mediated by the H_2 receptor as demonstrated by a wealth of pharmacological studies (Bottcher *et al*, 1989; Bazri and Dyeri, 1981; Dial *et al*, 1981). The effect of BA on basal and histamine -induced gastric acid secretion was studied. BA showed a dose-dependent reduction of gastric acid secretion. This compound shares the same five membered- ring structures of H_2 receptor antagonists with cimetidine, ranitidine and famotidine that are known to relieve or heal peptic ulcers (Yamada, 1996). Stimulation of gastric acid secretion by histamine is inhibited by BA, hence it may have competitive action by selectively inhibiting H_2-receptor antagonist as earlier reported by Hirschowitz and Molina (1983). There is therefore a good relationship between reduction of gastric acid secretion

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by BA and its anti-ulcer effect. To confirm the above assertion, BA was found to cause significant reduction and inhibition in mean ulcer score at high doses.

The mechanism of protection offered by mucus barrier shows that the more mucus produced, the lesser the degree of ulceration and also the mucus has a neutralizing effect on gastric acid (Zaidi and Mukerji, 1958). Several workers have reported that prostaglandins of the A, E and F types are anti-secretory agents. So also cytoprotective function of prostaglandins on gastric mucosa against injury or haemorrhagic lesions is by producing secretion which adheres to the mucosal surface of the stomach thereby acting as an unstirred layer that could allow a pH gradient to develop at the mucosal surface (Robert, 1975; Main and Whittle, 1977). Reports of the gastroprotective property of mucus have said that a decrease in gastric mucus renders the mucosa more susceptible to injury induced by various aggressive factors (Nosalova *et al*, 1991; Farre *et al*, 1995). The results showed that BA has a stimulatory effect on gastric mucus secretion and gastric mucus cells counts. This may apparently present a physical barrier against luminal pepsin and provide a stable unstirred layer that supports the surface neutralization of acid by mucosal bicarbonate (Allen, 1981). BA could be exhibiting anti-ulcer effect through its ability to cause the production of mucus and increasing mucus cells of the gastric mucosal. This in turn reduces the incidence of gastric ulcer and neutralization of gastric acid.

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The mode of action of BA may be similar to those of known drugs such as sucralfate, PAR-2 agonist, NO donors and misoprostol which are reported to increase gastric mucus production in vivo. Sucralfate has been reported to increase gastric mucus production in vivo (Slomiany *et al*, 1991) and in vitro (Takahashi and Okabe, 1996). It does this by increasing inositol triphospate (IP_3) content probably through the activation of phospolipase C, and the subsequent IP_3-elicited Ca^{2+} mobilization may be involved in the stimulatory effect of sucralfate (Slomiany *et al*, 1991). Misoprostol increases gastric mucus secretion by increasing the thickness of mucus layer, enhancing mucosal blood flow as a result of direct vasodilatation, stabilization of tissue lysosomes or vascular endothelium and improvement of mucosal regeneration capacity. PAR-2 agonist administered parenterally or orally triggers secretion of gastric mucus through the release of endogenous CGRP and tachkinnins. Allen (1981).reported that gastric mucus plays a key role in gastric cytoprotection by sustaining a significant pH gradient from the acid lumen (pH 1.5- 3.5) to the near neutral pH of the epithelial surface (6.7- 7.0), and also by inhibiting pepsinogen activation and pepsin back-diffusion. Mojzis *et al,* (1995) also suggested that gastric mucus is an important factor in gastric mucosal defense.

The involvement of oxygen derived free radicals such as the superoxide anion, hydrogen peroxide, and hydroxyl radicals are well established in the pathogenesis

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of ischaemic injury of gastrointestinal mucosa and in other models of mucosal damage induced by non-steroidal anti-inflammatory drugs (Joseph *et al*, 1999), ethanol (Simith *et al*, 1996), feeding restriction and stress (Yelken *et al*, 1999).

The pretreatment of animals with BA significantly decrease the MDA concentration compared to the control. In this study, measurement of gastric mucosal malondialdehyde concentration, which is the end-product of lipid peroxidation was performed and was found to be decreased. Levels of MDA are thought to reflect free radical mediated cell membrane damage (Demir *et al*, 2003). Also, the depletion of gastric mucosal glutathione (GSH) may result in the accumulation of free radicals that can initiate membrane damage by lipid peroxidation. Similar, increased level of GSH will cause diminished level of free radicals, and thus less damage to membrane by lipid peroxidation. It has been suggested that free radicals are closely related with peptic ulcer and gastritis (Salim, 1989). Oxygen free radicals are detrimental to the integrity of biological tissue and mediate their injury. The mechanism of damage involves lipid peroxidation, which destroys cell membranes with the release of intracellular components such as lysosomal enzymes, leading to further tissue damage. The free radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and DNA damage (Schraufstatter *et al*, 1988). Salim *et al*, (1994) investigated the

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influence of free radical scavengers on the healing gastric and duodenal ulcers resistant to therapy and found that antioxidative therapy stimulates the healing of therapy resistant ulcers. There is substantial evidence that oxygen derived free radicals play an important role in the pathogenesis of the injury of various tissues, including the digestive system (Santra *et al*, 2000; Choi *et al*, 1999). It is known that radical scavengers, such as alpha (α) tocopherol, carotenoids and glutathione redox system, play a significant role in protecting membranes from oxidation damage. The reduction of MDA concentration in this study may be due to the ability of BA to increase anti-oxidant activity. This therefore supports other studies that demonstrated a reduction in lipid peroxidation of the gastric mucosa shown to be associated with increased activities of anti-oxidant enzymes (Dela and Motilva, 1999; Melchiorri *et al*, 1997). Other studies have also shown that the protective activity of gastric mucus is due to the anti-oxidant activity conferred on it by its rich glycoprotein content (Cross *et al*, 1981; Oluwole and Saka, 2001). Allen (1981) has earlier reported that gastric mucus contains glycoproteins at concentrations as high as 50 mg/ml.

The histological slides from this study reveal more functional mucus cells with the surface mucus cells being more numerous and seemed to be disintegrating into spaces of the gastric pits in treated animals. Thus, there is a significant hyperplasia as shown. The histological observation justifies the results observed in the study on

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gastric mucus cell count. The hyperplasia observed could be due to the ability of BA to stimulate mucosal cell growth. It may also be due to increased synthesis of nitric oxide (NO) which normally causes vasodilatation of blood vessel, thus increasing mucosal blood flow.

CONCLUSION

Betulinic acid increases gastric mucus secretion, increases gastric mucus cells and decreases gastric acid secretion and reducing the level of MDA concentration i.e. increasing anti-oxidant activity via decreasing the level of free radical formation. These observations therefore justify the mechanisms underlying the anti=ulcer effect of betulinic acid. More studies are needed to shed more light in all these areas. It could be used as a promising new lead compound against gastric ulcer.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

References

Adams, J. M.; Cory, S. (2007): The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 26, 1324-1337.

Adekunle, A.A.; Duru, C. and Odufuwa, O.M. (2003): Antifungal activity and Phytochemical screening of the crude extracts of *khaya Ivorensis Juss* (Meliaceae) and *Tetracera potatoria* L. (Dilleniaceae) (2003): South Africa Journal of Botany. 69(4):566-571.

Adesanwo, J.K.; Ekundayo, O.; Oluwole, F.S.; Olajide, O.A.; Van Der Berge, A.J.J. and Findlay, J.A.(2003): The Effect Of *Tetracera Potatoria* and its constituent Betulinic Acid on Gastric Secretion and Experimentally-Induced Gastric Ulceration. Nig J Physiol Sci 18(1-2):21-26.

Aguwa C. and Okunji C.O.(1986). Gastrointestinal studies of *Pyrenacantha staudth* leaf extracts. J. Ethnopharmacol. 15:45-55.

Ahmet Aydin (2003):: Role of free radicals in peptic ulcer and gastritis. Turk J Gastroenterol; 14(1): 39-43.

Aierd J, Betall HN, Robert JAF (1953): Blood group in relation to peptic ulceration and carcinoma of colon, rectum and brinchus. Brit Med J 2: 315.

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

UNIVERSITY OF IBADAN LIBRARY

Alakurtti, S.; Makela, T.; Koskimies, S.; Yli-Kauhaluoma (2006): J. Pharmacological properties of the ubiquitous natural product betulin. Eur. J. Pharm. Sci., 29, 1-13.

Allen A (1981): Structure and function of gastrointestinal mucus. In: Physiology of the Gasstrointestinal Tract. LR Johnson (ed), New York, Raven Press, 1981.Pp 617-639

Allen A, Flemston G, Garner A, Kivilaakso C (1993): Gastroduodenal mucosal protection. Physio Reviews 73: 135-140.

Alphin RS and Ward JW (1967): Actions of hexapyronium bromide on gastric secretion in dogs and ulceration in rats. Arc inter de Pharmacology Therapy. 168: 82-100.

Andre, N.; Carre, M.; Brasseur, G.; Pourroy, B.; Kovacic, H.; Briand, C.; Braguer, D (2002): Paclitaxel targets mitochondria upstream of caspase activation in intact human neuroblastoma cells. FEBS Lett. 532, 256-260.

Baron JH (1963): Studies of basal and peak acid output with an augmented Histamine test. Ent 4: 136-144.

Batzri, S and Dyeri, J. (1981): Aminopyrine uptake by guinea pig gastric mucosa cells mediation by cyclic AMP and interactions among secretagogues. Biochem. Biophys. Acta 675: 416-462.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

Beaumont W (1833): Experiments and observations on the gastric juice and the physiology of digestion. Plattsburgh, NY. Allen.

Bejar E, Amarquaye A, Che C-T, Malone MH, Fong HHS (1995): Constituents of Byrsonima crassifolia and their spasmogenic activity. Int J Pharmacog 33:25–32.

Bottcher, G., Hakason, G., Seesalu, N.R. and Sundler, F. (1989): Effect of long hypergastrinaemia on the ultrastructure of enterochromaffin-like cells in the stomach of the rats, hamster and guinea pig. Cell Tissue Resp. 256: 247-257.

Bringmann G, Saeb W, Assi LA, Francois G, Narayanan ASS, Peters K, Peters EM. (1997): Betulinic acid: Isolation from Triphyophyllum peltatum and Ancistrocladus heyneanus, antimalarial acitivity, and crystal structure of the benzyl ester. Planta Med 63:255–257.

Carter, D.C. (1980): Aetiology Of Peptic Ulcer: In Scientific Foundation Of Gastroenterology. Pg 344. W.Sircus and A.N. Smith (Ed.). Heinemann,London.

Chang CW, Wu TS, Hsieh YS, Kuo SC, Chao PDL.(1999): Terpenoids of Syzygium formosanum. J Nat Prod 62:327–328.

Choi MA, Kim BS, Yu R (1999): Serum antioxidative vitamin levels and lipid peroxidation In gastric carcinoma patients. Cancer Lett; 136: 89-93.

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

UNIVERSITY OF IBADAN LIBRARY

Cole BJW, Bentley MD, Hua Y. (1991): Triterpenoid extractives in the outer bark of Betula lenta (black birch). Holzforschung 45:265–268.

Corney, S.J.; Morrissey, S.M. and Woods, R.J. (1974): A method for the quantitative estimation of gastric barrier mucus. J. Physiol. London. 242. Pg 116.

Cross CE, Halliwell B, Allen A (1984): Antioxidant protection : a function of tracheobronchoncial and gastrointestinal mucus. Lancet 328-330.

Cullen DJ, Hawkey GM, and Greenwood DC (1997): "Peptic ulcer bleeding in the elderly: relative roles of Helicobacter pylori and non-steroidal anti-inflammatory drugs". Gut 41 (4): 459–62.

Davenport HW (1970): Salicylate damage to the gastric mucosal barrier. N Eng J Med 276: 1307-1312.

Davies RE (1948): Hydrochloric acid production by isolated gastric mucosa. Biochem J 42: 609-621.

Degterev, A.; Boyce, M.; Yuan, J. (2003): A decade of caspases. Oncogene . 22, 8543- 8567.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

Dela Lastra, AC and Motilva MJ (1999): Protective effects of melatonin on indomethacin- induced gastric injury in rats. J Pineal Res 26:101-107.

Demir S, Yilmaz M, Köseoğlu M, Akalin N, Aslan D, Aydin A (2003): Role of free radicals in peptic ulcer and gastritis. Turk J Gastroenterol 14 (1): 39-43

Dial, E. Thompson, WJ and Rosenfeld, GC (1981): Isolated parietals cells histamine response and pharmacology. J. Pharmacol. Exp. Ther. 219:586-590.

Doll, R. (1962): Clinical trials of a Triterpenoid Liquorice compound in gastric and duodenal ulcer. Lancet 11: 793

Dunstan CA, Liu B, Welch CJ, Perera P, Bohlin L. (1998): Alphitol, a phenolic substance from Alphitonia zizyphoides which inhibits prostaglandin biosynthesis in vitro. Phytochemistry 48:495–497.

Durst, T.;Merali, Z.;Arnason, J.T.; Sanchez-Vindas, E.P.& Poveda Alvarez, L.J.(2002): Anxiolytic Marcgraviaceae Compositions Containing Betulinic Acid, Betulinic Acid Derivatives, and Methods.

Ehrhardt, H.; Fulda, S.; Fuhrer, M.; Debatin, K. M.; Jeremias, I. (2004): Betulinic acid induced apoptosis in leukemia cells. Leukemia 18, 1406-1412.

Elegbe RA (1974): Prevention by the anticholinergic drugs of indomethacin induced gastric ulceration in rats. Isreal J Med Sci 10:145

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

Elegbe RA (1978): Comparative studies on starvation and indomethacin induced gastric ulceration in albino rats. Biochem Exp Biol 14(2): 159-166.

Elegbe RA and Bamgbose SA (1976): Protective dietary factors in experimental ulceration study of some Nigerian cereal and tubers. Postgrad Med Journ 52:258-263.

Enwerem NM, Okogun JI, Wambebe CO, Okorie DA, Akah PA. (2001): Antihelmintic activity of the stem bark extracts of Berlina grandiflora and one of its active principles, betulinic acid. Phytomedicine 8:112–114.

Evers, M.; Poujade, C. And Solers, F. (1996): Betulinic Acid Derivatives: A new class of Human Immunodeficiency virus Type 1 specific inhibitors with a new mode of Action. J Med Chem. 39:1056-1068.

Flekhter, O.B.; Medvedeval,N.I; Karachurina, L.T.;Baltina, L.A.; Galin,F.Z.; Zarudi, F.S. & Tdstikor,G.A. (2005): Synthesis and Pharmacological Activity of Betulin, Betulinic Acid and Allobetullin Esters. Journal of Pharmaceutical Chemical. Vol 39, No. 8/ August.

Farre AJ, Colombo M, Alvarez I, Glavin GB (1995): Some novel 5-hydroxytryptamine 1A (5-HT_{1A}) receptor against reduce gastric acid and pepsin secretion, reduce experimental gastric mucosal injury and enhance gastric mucus in rats. J Pharmacol Exp Ther 272:832-837.

Freire SMF, Emim AJS, Lapa AJ, Souccar C, Torres LMB (1993): Analgesic and anti- inflammatory properties of scoparia. Phytother Res 7:408-414.

Freire, S.M., Baltina, L.A.; Galin,F.Z.; Zarudi, F.S. (1996): Sympathomimetic Effects of *Scoparia Dulcis L* and Catecholamines isolated from Plant Extracts. J Pharma Pharmacol 48(6): 624-628.

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

UNIVERSITY OF IBADAN LIBRARY

Fujioka T, Kashiwada Y, Kilkuskie RE, Cosentino LM, Ballas LM, Jiang JB, Janzen WP, Chen I-S, Lee KH (1994): Anti-AIDS agents. 11. Betulinic acid and platanic acid as anti-HIV principles from Syzigium claviflorum, and the anti-HIV activity of structurally related triterpenes. J Nat Prod 57:243–247.

Fulda S, Debatin KM (2000): Betulinic acid induces apoptosis through a direct effect on mitochondria in neuroectodermal tumors. Med Pediatr Oncol 35:616–618.

Fulda S, Friesen C, Los M, Scaffidi C, Mier W, Benedict M, Nunez G, Krammer PH, Peter ME, Debatin KM (1997): Betulinic acid triggers CD95 (APO-1/Fas)- and p53- independent apoptosis via activation of caspases in neuroectodermal tumors. Cancer Res 57:4956–4964.

Fulda S, Jeremias I, Steiner HH, Pietsch T, Debatin KM. (1999): Betulinic acid: A new cytotoxic agent against malignant brain-tumor cells. Int J Cancer 82:435–441.

Fulda S, Scaffidi C, Susin SA, Krammer PH, Kroemer G, Peter ME, Debatin K-M (1998): Activation of mitochondria and release of mitochondrial apoptogenic factors by betulinic acid. J Biol Chem 273: 33942–33948.

Fulda, S.; Susin, S. A.; Kroemer, G.; Debatin, K. M.(1998): Molecular ordering of apoptosis induced by anticancer drugs in neuroblastoma cells. Cancer Res. 58, 4453-4460.

Galgon T, Hoke D, Drager B.(1999): Identification and quantification of betulinic acid. Phytochem Anal10:187–190.

Ghosh, M.N. and Schild, H.O. (1958): Continuous recording of gastric acid secretion in rat. Brit. J. Pharmacol. 21: 1396.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

Gregory RA, Tracy HJ, Agarwal KL and Grossman MI (1967): Amino acid constitution of two gastrins isolated from Zollinger- Ellison tumor tissue. Gut 10: 603-608.

GI Consult: Perforated Peptic Ulcer (2007). http://www.emedmag.com/html/pre/gic/consults.

Gutteridge, JMC and Wilkins, C (1982): Copper dependent hydroxyl radical damage to ascorbic acid: Formation of a thiobarbituric acid reactive product. FEBS Letters 137:327-340.

Hayek EWH, Jordis U, Moche W, Sauter F. (1989): A bicentennial of betulin. Phytochemistry 28:2229–2242.

Hersey SJ and Sachs G (1995): Physiological reviews. Vol 75. No 1.

Higa M, Ogihara K,Yogi S.(1998): Bioactive naphthoquinone derivatives from Diospyros maritime Blume. Chem Pharm Bull 46:1189–1193.

Hirschowitz, B.I. and Molina, E. (1983). Effect of four H2- histamine antagonists on bethamecol-stimulated acid and pepsin secretion I in the dog. J. Pharmacol. Exp. Ther. 224, 341-345.

Hollander FF (1954): The two components mucous barrier its activity in protecting the gastrointestinal mucosa against peptic ulceration. Arch Inter Med 93: 107.

Huguet A-I, Recio MC, Manez S, Giner RM, Rios J-L (2000): Effect of triterpenoids on the inflammation induced by protein kinaseCactivators, neuronally acting irritants and other agents. Eur J Pharmacol 410:69–81.

Ikuta A, Kamiya K, Satake T, Saiki Y.(1995): Triterpenoids from callus tissue cultures of Paeonia species. Phytochemistry 38:1203–1207.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

Ivy AC, Grossman MI and Bachrach WH (1950): Peptic ulcer, 27. Blakiston Philadelphia, Pa.

Ivy AC (1944): The mechanism of gastric secretion. Surgery 10: 861-878.

Jagadeesh SG, Krupadanam GLD, Srimannarayana G, Murthy SS, Kaur A, Raja SS (1998): Tobacco caterpillar antifeedant from the gotti stem wood triterpene betulinic acid. J Agric Food Chem 46:2797–2799.

Johannessen T.(2007). "Peptic ulcer disease". Pasienthandboka.

Joseph RM, Varela V, Kanji VK,(1999): Protective effects of zinc in indomethacin- Induced gastric mucosal injury: evidence

Kamiya K, Yoshioka K, Saiki Y, Ikuta A, Satake T (1997): Triterpenoids and flavonoids from Paeonia lactiflora. Phytochemistry 44:141–144.

Karen Liby,Tadashi Honda, Charlotte R.W., Renee Risingsong, Darlene B. Royce, Nanjoo Suh, Albena T. Dinkova-Kostova, Katherine K. Stephenson, Paul Talalay, Chitra Sundararajan, Gordon W.G. and Micheal B.S. (2007): Novel semisynthetic analogues of betulinic acid with diverse cytoprotective, antiproliferative, and proapoptotic activities. Molecular Cancer Ther. 6: 2113-2119.

Kashiwada Y, Wang HK, Nagao T, Kitanaka S, Yasuda I, Fujioka T, Yamagishi T, Cosentino LM, Kozuka M, Okabe H, IkeshiroY, Hu CQ,Yeh E, Lee KH. (1998): Anti- AIDS agents. 30. Anti-HIV activity of oleanolic acid, pomolic acid, and structurally related triterpenoids. J Nat Prod 61:1090–1095.

Kim DSHL, Chen Z, Nguyen VT, Pezzuto JM, Qui S, Lu Z-Z.(1997): A concise semi- synthetic approach to betulinic acid from betulin. Synth Commun;27:1607–1612.

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

UNIVERSITY OF IBADAN LIBRARY

Kinoshita K, Akiba M, Saitoh M,YeY,Koyama K, Takahashi K,Kondo N,Yuasa H. (1998): Antinociceptive effect of triterpenes from cacti. Pharm Biol 36:50–57.

Kwon HJ, Shim JS, Kim JH, ChoHY,YumYN, Kim SH,Yu J (2002): Betulinic acid Inhibits growth factor-induced in vitro angiogenesis via the modulation of mitochondrial function in endothelial cells. Jpn J Cancer Res 93:417–425.

Li Ma, Piero Del Soldato and John L. (2002): Divergent effects of new cyloxygenase inhibitors on gastric ulcer healing: Shifting the angiogenic balance. Pharmacology. Vol. 99. No 20. Pg 13243 - 13247.

Lin HC, Ding HY, Wu YC. (1998): Two novel compounds from Paeonia suffruticosa. J Nat Prod 61:343–346.

Ma J, Starck SR, Hecht SM. (1999): DNA polymerase b inhibitors from Tetracera boiviniana. J Nat Prod 62:1660–1663.

Macias FA, Simonet AM, Galindo JCG. (1995): Natural products that influence plant growth and development from plants: The case of Melilotus messanensis (L.) All. Proc. Growth Reg Soc Am 22:53–57.

Main IHM and Whittle BJ (1975): Investigation of the vasodilator and anti secretory role of prostaglandins in the rat gastric mucosa by the use of non-steroidal anti- inflammatory drugs. Br J Pharmacol 53: 217.

Mayaux, J.F.; Bousseau, A.; Panwels, R.; De Clerq, E. And Pecq, J.B. (1994): Triterpene derivatives that blocks the entry of Human Immunodeficiency Virus Type I into Cells. Proc Natl Acad Sci USA 91:3564 -3568.

M$^{\underline{C}}$ Quaid KR and Isenberg JI (1992): Medical therapy of peptic ulcer disease. Surg Clin North Am 72(2): 285-316.

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

UNIVERSITY OF IBADAN LIBRARY

Melzig MF, Bormann H (1998): Betulinic acid inhibits aminopeptidase N activity. Planta Med 64:655–657.

Melchiorri D, Sewerynek E, Reiter RJ, Ortiz GG, Poeggeler B and Nistisco G. (1997): Suppressive effect of melatonin administration on ethanol –induced gastroduodenal injury in rats in vivo. Br J Pharmacol 264-270.

Meng, R. D.; El-Deiry, W. S. (2001): p53-independent upregulation of KILLER/DR5 TRAIL receptor expression by glucocorticoids and interferon-gamma. Exp. Cell Res. 262,154-169.

Menguy R and Desbaillet L (1967): Role of inhibition of gastric mucous secretion in the Phenomenon of gastric mucosal injury by indomethacin. A M J Dig 12: 862-866.

Micheau, O. (2003): Cellular FLICE-inhibitory protein: an attractive therapeutic target? Expert Opin. Ther. Targets. 7, 559-573.

Mojzis J, Kohut A, Mirossay L, Nicak, Pomfy M, Benicky M, Bodnar J (1995): Effect of sucralfate on ischemia/reperfusion-induced gastric mucosal injury. Slovakofarma Rev 5:53-57.

Mukherjee R, Jaggi M, Rajendran P, Srivastava SK, Siddiqui MJA, Vardhan A, Burman AC (2004) Synthesis of 3-O-acyl/3-benzylidene/3-hydrazone/3-hydrazine/17-carboxyacryloyl ester derivatives of betulinic acid as anti-angiogenic agents. Bioorg. Med.Chem. Lett.**,** 14, 3169-3172.

National Digestive Diseases Information Clearing house (2009). For nearly 100 years, scientists and doctors thought that ulcers were caused by stress, spicy food, and alcohol.

Newman, D. J.; Cragg, G. M.; Snader, K. M.(**2003**) Natural products as sources of new Drugs over the period 1981-2002. *J. Nat. Prod.***,** *66*, 1022-1037.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

Njar VCO, Adesanwo JK, Makinde JM, Taiwo OB (1994): Anti-ulcer activity of stem bark extract of *entradophiagm anglolense* . Phytotherapy Research. Vol 8:46-48.

Nick A, Wright AD, Rali T, Sticher O (1995). Antibacterial triterpenoids from Dilleni papuana and their structure–activity relationships. Phytochemistry 40:1691–1695.

Noda Y, Kaiya T,Kohda K, KawazoeY (1997): Enhanced cytotoxicity of some triterpenes toward leukemia L1210 cells cultured in lowpHmedia: Possibly a newmode of cell killing.Chem Pharm Bull 45:1665–1670.

Nosalova V, Juranek I, Babulova A (1991): Effect of pentacaine and ranitidine on gastric mucus changes induced by cold-restraint stress in rats. Agent Action 33: 164-166.

O'Connell MM, Bently MD, Campbell CS, Cole BJW. (1988):Betulin and lupeol in bark from four white-barked birches. Phytochemistry 27:2175–2176.

Oluwole FS and Saka MT (2001): Effect of thyroid hormone on gastric mucus secretion and indomethacin-induced gastric ulcers in rats. J Med Sci 2007. Pg 1-4.

Oluwole FS, Ayo JA, Omolaso BO, Emikpe BO and Adesanwo JK (2008): Methanolic extract of *Tetracera Potatoria,* an anti-ulcer agent increases gastric mucus secretion and endogenous antioxidants. Nig J Physiol Sci Vol 23: 1-2. Pg 79-83.

Parmar, N.S. and Ghosh, M.N. (1981): Gastric anti-ulcer activity of (+)-cyanidanol-3, a histidine decarboxylase inhibitor. Eur. J. Pharmacol. 69: 25-32.

Pavlov, I (1910): The work of the digestive glands. London, Griffin.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

Pathak A, Singh SK, Biabani MAF, Kulshreshtha DK, Puri SK, Srivastava S, Kundu B (2002): Synthesis of combinatorial libraries based on terpenoid scaffolds. Combin Chem High Throughput Screen 5:241–248. Phytomedicine;4:309–313.

Peptic Ulcer:Peptic Disorders: Merck Manual Home Edition (2007). http://www.merck.com

Pezzuto JM, Schmidt ML, Kuzmanoff KL, Ling-Indeck L (1995): Betulinic acid induces apoptosis in human neuroblastoma cell lines. Eur J Cancer 33:2007–2010.

Pisha E, Chai H, Lee IS, Chagwedera TE, Farnsworth NR, Cordell AC, Beecher CWW, Fong HHS, Kinghorn AD, Brown DM, Wani MC, Wall ME, Hieken TJ, Das Gupta TK, Pezzuto JM. (1995): Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. Nat Med1:1046–1051.

Purohit MC, Rawat MSM (1993): Pant G. Spermicidal activity and chemical analysis of Schefflera venulosa. Fitoterapia 64:274–275.

Raisova M, Hossini AM, Eberle J (2001): "The Bax/Bcl-2 ratio determines the susceptibility of human melanoma cells to CD95/Fas-mediated apoptosis". The Journal of Investigative Dermatology 117 (2): 333–40.

Rao CV, Sairam K, Goel RK (2000): Experimental evaluation of Bacopa monniera on rat gastric ulceration and secretion. Indian J Physiol Pharmacol. Oct; 44(4): 435-441

Rieber M, Strasberg M (1998). "Induction of p53 without increase in p21WAF1 in betulinic acid-mediated cell death is preferential for human metastatic melanoma". DNA and Cell Biology 17 (5): 399–406

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

Recio MC, Giner RM, Manez S, Gueho J, Julien HR, Hostettmann K, Rios JL.(1995): Investigations on the steroidal anti-inflammatory activity of triterpenoids from Diospyros leucomelas. Planta Med 61:9–12.

Roberts EI (1977): An intestinal disease produced experimentally by a prostaglandins deficiency. Gastroenterol 69: 1045-1047.

Ryu SY, Lee CK, Ahn JW, Lee SH, Zee OP (1993): Antiviral activity of triterpenoid derivatives. Arch Pharm Res 16:339–342.

Ryu SY, Lee CK, Lee CO, Kim HS, Zee OP. (1992): Antiviral triterpenes from Prunella vulgaris. Arch Pharm Res 15:242–245.

Ryu SY, Oak M-H, Yoon S-K, Cho D-I, Yoo G-S, Kim T-S, Kim K-M. (2000): Anti- Allergic and anti-inflammatory triterpenes from the herb of Prunella vulgaris. Planta Med 66:358–360.

Saelens, X.; Festjens, N.; Vande Walle, L.; van Gurp, M.; van Loo, G.; Vandenabeele, P. (2004): Toxic proteins released from mitochondria in cell death. Oncogene 23, 2861- 2874.

Safayhi H, Sailer E-R (1997): Anti-inflammatory actions of pentacyclic triterpenes. Planta Med 63: 487– 493.

Sairam K, Rao CV, Babu MD, Goel RK (2001): Prophylactic and curative effects of Bacopa monniera in gastric ulcer models. Phytomedicine, Nov; 8(6): 423-430.

Salim AS (1989): Scavenging free radicals to prevent stress induced gastric mucosal injury. Lancet; 1390.

Salim AS (1994): Role of free radical scavengers in the management of refractory duodenal ulceration. A new approach. J Surg Res; 56: 45-52.

Salti GI, Kichina JV, Das Gupta TK, Uddin S, Bratescu L, Pezzuto JM, Mehta RG, Constantinou AI (2001): Betulinic acid reduces ultraviolet-C-induced

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

UNIVERSITY OF IBADAN LIBRARY

DNA breakage in congenital melanocyte naeval cells: Evidence for a potential role as a chemopreventive agent. Melanoma Res 11:99–104.

Santra A, Chowdhury A, Chaudhuri Sl (2000): Oxidative stress in gastric mucosa in helicobacter pylori infection. Indian J Gastroenterol; 19: 21-3.

Schmassmann A, Waser B, Flogerzi B and Reubi J (2005): Expression of functional neurokinin-I receptors in regenerative glands during gastric wound healing in rodents. Gastroenterology 126:784-795.

Schmidt, M.L.; Kuzmanoff, K.L.; Ling-Indeck, L. and Pezzuto, J.M. (2007): Betulinic Acid Induces Apoptosis in Human Neuroblastoma Cell Lines. European Journal of Cancer 33(12).

Schraufstatter I, Hyslop PA, Jackson JH, Cochrane CG (1988): Oxidant induced DNA damage of target celle. J Clin Invest 82:1040-50.

Schuhly W, Heilmann J, Calis I, Sticher O.(1999): New triterpenoids with antibacterial activity from Zizyphus joazeiro. Planta Med 65:740–743.

Selzer E, Pimentel E, Wacheck V, Schlegel W, Pehamberger H, Jansen B, Kodym R (2000): Effects of betulinic acid alone and in combination with irradiation in human Melanoma cells. J Invest Dermatol 114(5):935–940.

Selzer, E.; Thallinger, C.; Hoeller, C.; Oberkleiner, P.; Wacheck, V.; Pehamberger, H.; Jansen, B.(2002): Betulinic acid-induced Mcl-1 expression in human melanoma—mode of action and functional significance. Mol. Med. 8, 877-884.

Setzer, W.N.; Setzer, M.C.; Bates, R.B. And Jackes, B.R. (2000): Biologically Active Triterpenoids of Syncarpia Glomulifera Bark Extract from Paluma, North Queensland Australia. Planta Med 66:176–177.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

Shin YG, Cho KH, Chung SM, Graham J, Das Gupta TK, Pezzuto JM (1999):Determination of betulinic acid in mouse blood, tumor and tissue homogenates by liquid chromatography-electrospray mass spectrometry. J. Chromatogr. B Biomed. Sci. Appl.**,**732, 331-336.

Simith GS, Mercer DW, Cross JM (1996): Gastric injury induced by ethanol and ischemia- reperfusion in the rat. Differing roles for lipid peroxidation and oxygen radicals. Dig Dis Sci; 41: 1157-64.

Singh P, Sharma S. (1997): Triterpenoid constituents of the seed of Diospyros melanoxylon,Tecomella undulata, and Terminalia bellirica. J Indian Chem Soc 174:504–505.

Slomiany BL, Piotrowski J, Tamura S, Slomiany A (1991): Enhancement of the protective qualities of gastric mucus by sucralfate: role of phosphoinositides. Am J Med: S30-S36.

Sofowora, A.(1982): Historical review of Traditional Medicine in Medicinal plants and Traditional medicine in Africa. John Wiley and Son Ltd. New York. In association with Spectrum Books Ltd. Ibadan, Nigeria. Pg 9-25.

Somova Li, Shode F.O., Ramnanan P., Nadar A.(2003). Anti-hypertensive, ant- atherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea* a subspecies Africana leaves. J. Ethnopharmacol. Feb 8, 4 (2-3): 299-305.

Sun HH, Kaplita PV, Houck DR, Stawicki MB, McGarry R, Wahl RC, Gillum AM, Cooper R. (1996): A metalloproteinase inhibitor from Doliocarpus verruculosus. Phytother Res 10:194–197.

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Syrovets T, Buchele B, Gedig E, Slupsky JR, Simmet T (2000): Acetyl-boswellic acids are novel catalytic inhibitors of human topoisomerase I and IIa. Mol Pharm 58:71–81.

Takahashi S. and Okabe S. (1996): Stimulatory effects of sucralfate on secretion and Synthesis of mucus by rabbit mucosal cells: Involvement of phospholipase C. Dig Dis Sci. : 498-504.

Takeoka G.L., Dao R., Teranishi R., Wong S., Flessa L., Harden, R., Edwards R. (2000): Identification of three triterpenoids in almond hulls. J Agric Food Chem 48: 3437- 3439.

Tan, Y.; Yu, R. And Pezzuto, J.M. (2003): Betulinic Acid Induced Programmed Cell Death In Human Melanoma Cells involves Mitrogen- Activated Protein Kinase Activation. Clin Cancer Res 9(7):2866-2875.

Thurnher, D.; Turhani, D.; Pelzmann, M.; Wannemacher, B.; Knerer, B.; Formanek, M.; Wacheck, V.; Selzer, E. (2003): Betulinic acid **:** a new cytotoxic compound against malignant head and neck cancer cells. Head & Neck 25, 732-740.

Vane JR (1971): Inhibition of prostaglandin synthesis as a mechanism of action of aspirin Like drugs. Nature New Bio 231-232.

Wachirawat W, Hanucharurnkul S, Suriyawongpaisal P, (2003). "Stress, but not Helicobacter pylori, is associated with peptic ulcer disease in a Thai population". J Med Assoc Thai 86 (7): 672–85.

Wachsbeberger PR, Burd R,Wahl ML, Leeper DB (2002): Betulinic acid sensitization of low pH adapted human melanoma cells to hyperthermia. Int J Hyperthermia 18:153–164.

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Walczak, H.; Krammer, P. H. (2000): The CD95 (APO- 1/Fas) and the TRAIL (APO-2L) apoptosis systems. Exp. Cell Res. 256, 58-66.

Walsh J and Grossman S (1975): Medical progress gastrin. N. Engl J Med 292: 1324-1332.

Wang BH, Polya GM (1996): Selective inhibition of cyclic AMP-dependent protein kinase By amphiphilic triterpenoids and related compounds. Phytochemistry 41:55–63.

Wick, W.; Grimmel, C.; Wagenknecht, B.; Dichgans, J.; Weller, M. (1999): Betulinic acid**-** induced apoptosis in glioma cells: A sequential requirement for new protein synthesis, formation of reactive oxygen species, and caspase processing. J. Pharmacol. Exp.Ther. 289, 1306-1312.

Wikipedia, the free encyclopedia (2008)*:* http://en.wikipedia.org/wiki/betulinic acid.

Yamada, T. (1996). Textbook of Gastroenterology. Vol.1. J.B. Lippincott Company. Pg 1266-1268.

Yasukawa K, Takido M, Matsumoto T, Takeuchi M, Nakagawa S (1991): Sterol and triterpene derivatives from plants inhibit the effects of a tumor promoter, and sitosterol and betulinic acid inhibit tumor promotion in mouse skin two-stage carcinogenesis. Oncology;48:72–76.

Yasukawa K, Yu SY, Yamanouchi S, Takido M, Akihisa T, Tamura T (1995): Some lupane- type triterpenes inhibit tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. Phytomedicine 4:309–313.

Yelken B, Dorman T, Erkasap S,(1999): Clonidine pretreatment inhibits stress-induced gastric ulcer in rats. Anesth Analg;89: 159-62.

AFRICA DIGITAL HEALTH REPOSITORY LIBRARY

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Zaidi SH and Mukerji B (1958): Experimental peptic ulcer (Part 1). The significance of mucous barrier. Ind J Med Res 46:27-31.

Zhang M.Z., Liu L., and Wang S.R. (2001). Experimental study of protective effect of longximuo capsule on isoproterenol myocardial injury in rats. Zhongguo Zhong Xi Yi Jie He Za Zhi 21: 51-53.

Zhu M, Phillipson JD, Greengrass PM, Bowery NG. (2000): Chemical and biological investigation of the root bark of Clerodendrum mandarinorum. Planta Med 62:393–396.

Zuco V, Supino R, Righetti SC, Cleris K, Marchesi E, Gambacorti-Passerini C, Formelli F (2002): Selective cytotoxicity of betulinic acid on tumor cell lines, but not normal cells. Cancer Lett 175:17–25.

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APPENDIX 1

The Effect of Betulinic Acid (BA) On Indomethacin Induced Gastric Ulceration in Male Albino Rats

Treatment	***Animal***	***Ulcer score***
Normal	I	8.0
Saline + DMSO)	II	7.0
(Control)	III	7.0
	IV	6.0
	V	7.0
	VI	6.0
	VII	8.0
	VIII	7.0
	Mean Ulcer index	**7.0 ± 0.27**

BA = Betulinic Acid

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Treatment	*Animals*	*Ulcer Index*
BA	I	5.0
(0.5 $mgkg^{-1}$)	II	6.0
	III	6.0
	IV	5.0
	V	6.0
	VI	8.0
	VII	6.0
	VIII	8.0
	Mean Ulcer index	**6.25 ± 0.42**

BA = Betulinic Acid

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Treatment	*Animal*	*Ulcer Index*
BA(1.5 mgkg^{-1})	I	1.0
	II	0.5
	III	0.5
	IV	0.5
	V	1.0
	VI	1.0
	VII	1.0
	VIII	0.5
	Mean Ulcer index	**0.75 ± 0.095**

BA = Betulinic Acid

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Treatment	*Animal*	*Ulcer Index*
BA (3.0 $mgkg^{-1}$)	I	0.5
	II	0
	II	0
	IV	0
	V	0
	VI	0.5
	VII	0.5
	VIII	0
	Mean Ulcer index	**0.25 ± 0.094**

BA = Betulinic Acid

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APPENDIX II

Effect of Betulinic Acid on Gastric Mucus Secretion

Control

	Weight of Stomach (g)	*Absorbance at 602 nm*	*Weight of Dye (mg x 10^{-2})*	*Gastric Mucus = Weight of Dye / Weight of Stomach (mg/g tissue x 10^{-2})*
1	**1.92**	**0.475**	**0.090**	**4.69**
2	**2.02**	**0.375**	**0.069**	**3.41**
3	**1.88**	**0.475**	**0.090**	**4.79**
4	**2.10**	**0.500**	**0.100**	**4.76**
5	**1.15**	**0.250**	**0.057**	**4.96**
6	**2.05**	**0.375**	**0.075**	**3.66**
7	**1.95**	**0.400**	**0.080**	**4.10**
8	**1.85**	**0.425**	**0.085**	**4.60**
			Total mean ± SEM	**4.40 ± 0.20**

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0.5mg/kg BA

	Weight of Stomach (g)	Absorbance at 602 nm	Weight of Dye (mg x 10^{-2})	Gastric Mucus = Weight of Dye / Weight of Stomach (mg/g tissue x 10^{-2})
1	1.54	0.375	0.075	4.87
2	1.27	0.200	0.048	3.78
3	1.68	0.425	0.087	5.18
4	1.75	0.375	0.077	4.40
5	1.14	0.200	0.050	4.39
6	1.36	0.250	0.060	4.41
7	1.82	0.425	0.086	4.73
8	1.90	0.425	0.095	4.97
			Total mean ± SEM	4.60 ± 0.16

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1.5 mg/kg BA

	Weight of Stomach (g)	Absorbance at 602 nm	Weight of Dye (mg x 10^{-2})	Gastric Mucus = Weight of Dye / Weight of Stomach (mg/g tissue x 10^{-2})
1	1.72	0.450	0.090	5.23
2	1.75	0.500	0.100	5.71
3	1.94	0.475	0.089	4.64
4	2.04	0.500	0.100	4.90
5	1.55	0.400	0.080	5.30
6	1.63	0.300	0.065	3.68
7	1.78	0.425	0.085	4.78
8	1.87	0.500	0.100	5.35
			Total mean ± SEM	4.90 ± 0.22

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3.0 mg/kg BA

	Weight of Stomach (g)	Absorbance at 602 nm	Weight of Dye (mg x 10^{-2})	Gastric Mucus = Weight of Dye / Weight of Stomach (mg/g tissue x 10^{-2})
1	1.62	0.425	0.085	5.25
2	1.70	0.475	0.095	5.59
3	1.82	0.475	0.090	4.95
4	1.95	0.475	0.095	4.87
5	1.98	0.500	0.100	5.05
6	1.57	0.425	0.085	5.41
7	1.78	0.475	0.090	5.06
8	1.86	0.500	0.100	5.40
			Total mean ± SEM	5.20 ± 0.09

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APPENDIX III

Effect of Betulinic Acid on Gastric Mucus Cell Count/mm^2

Control

Rat	1	2	3	4	5	Mean/mm^2
1	44	40	45	42	46	43.4
2	38	43	47	35	41	40.8
3	42	46	34	31	36	37.8
4	42	37	48	46	43	43.2
5	40	45	33	46	37	40.2
6	47	44	41	32	35	39.8
7	43	48	34	51	37	42.6
8	34	43	46	47	45	43.0
					Total ± Mean	41.4 ± 0.72

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0.5 mg/kg BA

Rat	1	2	3	4	5	Mean/mm^2
1	53	41	37	47	55	46.6
2	46	35	32	54	58	45.0
3	30	47	36	42	45	40.0
4	54	42	46	48	40	46.0
5	41	49	37	46	52	45.0
6	39	43	51	35	47	43.0
7	49	55	42	52	36	46.8
8	57	38	43	40	34	42.4
					Total ± Mean	44.4 ± 0.84

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1.5 mg/kg BA

Rat	1	2	3	4	5	Mean/mm^2
1	51	46	48	42	54	48.2
2	53	55	37	41	47	46.6
3	35	43	45	52	42	43.4
4	47	40	53	46	55	48.2
5	56	32	39	44	42	42.6
6	51	49	43	40	54	47.4
7	44	41	50	37	48	44.0
8	38	52	46	55	43	46.6
					Total ± Mean	45.9 ± 0.79

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3.0 mg/kg BA

Rat	1	2	3	4	5	Mean/mm^2
1	57	46	62	51	48	52.8
2	64	62	55	58	61	60.0
3	53	64	47	45	56	53.0
4	61	58	54	49	46	53.6
5	47	53	57	51	45	50.6
6	55	60	56	46	54	54.2
7	55	53	57	45	48	51.6
8	59	68	53	47	57	56.8
					Total ± Mean	54.1 ± 1.07

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APPENDIX 4

Effect of Betulinic Acid on Gastric Acid Secretion

Time (min)	Control	0.5 mg/kg BA	1.5 mg/kg BA	3.0 mg/kg BA
10	2.21± 0.052	2.10±0.073	1.35± 0.054	0.7500±0.042
20	2.14± 0.032	2.05±0.120	1.26± 0.057	0.7600 ±0.018
30	2.23± 0.059	1.95±0.060	1.40± 0.080	0.7300 ±0.037
40	2.19± 0.035	2.03±0.075	1.30± 0.038	0.7500 ±0.052
50	2.31± 0.023	2.14±0.057	1.38± 0.070	0.6900 ±0.023
70	3.23± 0.065	1.88±0.101	1.03± 0.053	0.5600 ±0.018
80	3.33± 0.041	1.81±0.040	1.06± 0.046	0.5500 ±0.027
90	3.23± 0.053	1.88±0.068	1.05± 0.078	0.5900 ±0.030
100	3.33± 0.049	1.83±0.045	1.16± 0.068	0.6100 ±0.040
110	3.29± 0.044	1.96±0.050	1.10± 0.066	0.5800 ±0.031

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Summary of the Effect of Betulinic Acid on Gastric Acid Secretion

Drug Dose (mg/kg)	Time (minutes)								
	10	20	30	40	50	70	80	90	100
Normal Saline (n=8)	2.21± 0.052	2.14 ± 0.032	2.23 ± 0.059	2.19 ± 0.035	2.31 ± 0.023	3.23 ± 0.065	3.33 ± 0.041	3.23 ± 0.053	3.33 ± 0.049
BA 0.5 (n=8)	2.10 ± 0.073	2.05 ± 0.12	1.95 ± 0.060	2.03 ± 0.075	2.14 ± 0.057	1.88 ± 0.10	1.81 ± 0.040	1.88 ± 0.068	1.83 ± 0.045
BA 1.5 (n=8)	1.35 ± 0.054	1.26 ± 0.057	1.40 ± 0.080	1.30 ± 0.037	1.38 ± 0.070	1.03 ± 0.053	1.06 ± 0.046	1.05 ± 0.078	1.16 ± 0.068
BA 3.0 (n=8)	0.75 ± 0.042	0.76 ± 0.018	0.73 ± 0.037	0.75 ± 0.51	0.69 ± 0.023	0.56 ± 0.18	0.55 ± 0.027	0.59 ± 0.030	0.61 ± 0.040

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APPENDIX V

Effect of Betulinic Acid on Malonialdehyde (MDA) Concentration

Control

Rat	Weight of Stomach (g)	Absorbance at 532 nm	MDA concentration (µmol/L x 10^{-6})
1	2.03	0.429	2.75
2	2.15	0.456	2.92
3	1.78	0.407	2.61
4	1.92	0.396	2.54
5	1.87	0.379	2.43
6	1.84	0.339	2.17
7	1.98	0.360	2.31
8	2.21	0.484	3.10
Mean ± SEM	1.97 ± 0.053	0.41 ± 0.017	2.60 ± 0.11

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0.5mg/kg BA

Rat	Weight of Stomach (g)	Absorbance at 532 nm	MDA concentration (µmol/L x 10^{-6})
1	1.82	0.260	1.67
2	2.12	0.332	2.13
3	1.93	0.243	1.56
4	2.31	0.270	1.73
5	2.25	0.329	2.11
6	1.78	0.318	2.04
7	1.89	0.287	1.84
8	2.17	0.275	1.76
Mean ± SEM	2.03 ± 0.072	0.289 ± 0.012	1.86 ± 0.076

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1.5 mg/kg BA

Rat	Weight of Stomach (g)	Absorbance at 532 nm	MDA concentration (µmol/L x 10^{-6})
1	1.88	0.251	1.61
2	1.95	0.300	1.92
3	2.11	0.237	1.52
4	1.74	0.315	2.02
5	1.83	0.287	1.84
6	2.03	0.292	1.87
7	1.67	0.278	1.78
8	1.78	0.328	2.10
Mean ± SEM	1.87 ± 0.053	0.29 ± 0.011	1.83 ± 0.069

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3.0 mg/kg BA

Rat	Weight of Stomach (g)	Absorbance at 532 nm	MDA concentration (µmol/L x 10^{-6})
1	2.21	0.204	1.31
2	1.76	0.173	1.11
3	1.92	0.164	1.05
4	1.87	0.187	1.20
5	2.07	0.144	0.92
6	1.87	0.136	0.87
7	1.73	0.200	1.28
8	2.18	0.168	1.08
Mean ± SEM	1.95 ± 0.065	0.17 ± 0.0087	1.10 ± 0.056

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