# MECHANISMS OF THE ANTI-GASTRIC ULCER ACTIVITY OF RISPERIDONE IN MALE RATS


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A THESIS IN THE DEPARTMENT OF PHYSIOLOGY

Submitted to the

FACULTY OF BASIC MEDICAL SCIENCES COLLEGE OF MEDICINE

In partial fulfillment of the requirement for the award of degree of

DOCTOR OF PHILOSOPHY OF THE UNIVERSITY OF IBADAN

FEBRUARY 2015

## ABSTRACT

Gastric ulcer and its treatment is a global problem, thus the search for a novel drug becomes a continuous one. Risperidone, though, anti-psychotic has been found to exhibit anti-ulcer activity, however, its mechanisms of action are yet to be fully elucidated. The study was designed to investigate the mechanisms underlying its anti-ulcer activity in rats.

Three hundred and thirty-six male Wistar rats (180-210 g) were divided into 5 groups of 96, 48, 96, 64 and 32 rats respectively and treated orally for 21 days. Ninety-six of them were divided into 3 sub-groups to be induced with ulcer using indomethacin, starvation and Water Immersion Restraint Stress (WIRS) methods, Each sub-group was pre-treated with distilled water (control) and risperidone (0.1, 0.3, 0.5 mg/kg). Forty-eight rats were further divided into 6 groups; risperidone (0.5 mg/kg), indomethacin (40mg/kg), cyclooxygenase-1 inhibitor (SC-560, 40mg/kg), cyclooxygenase-2 inhibitor (celecoxib, 15 mg/kg), celecoxib + SC-560 and celecoxib + SC-560 + risperidone. Gastric ulcers were scored using standard techniques. Ninety-six rats were divided into 3 sub-groups, with each group treated with distilled water and risperidone (0.1, 0.3, 0.5 mg/kg) and were assessed for Gastric Acid Secretion (GAS) induced by histamine, pentagastrin and carbachol using continuous perfusion techniques. Sixty-four rats were divided into 2 sub-groups and treated with distilled water and risperidone (0.1, 0.3, 0.5 mg/kg) for the assessment of Gastric Mucus Secretion (GMS) and Gastric Mucus Cell Counts (GMCC) using spectrophotometry and calibrated microscopy respectively. Thirty two rats divided into four treatment groups; distilled water and risperidone (0.1, 0.3, 0.5 mg/kg) were used for determination of malondialdehyde level by spectrophotometry. Histological studies on stomach tissues were done after staining with H&E and PAS stains using light microscope. Data were analysed using Student's t-test and ANOVA at $p = 0.05$.

Risperidone caused a significant dose-dependent reduction in gastric ulcer scores [0.1mg/kg (3.5±0.2), 0.3mg/kg (1.9±0.3), 0.5mg/kg (1.2±0.2)] compared with control (5.6±0.3) in WIRS; [0.1mg/kg (4.0±0.3), 0.3mg/kg (2.3±0.2), 0.5mg/kg (1.8±0.2)] compared with control

(6.1 ± 0.3) in starvation and [0.1mg/kg (4.9±0.3), 0.3 mg/kg (2.0±0.2), 0.5 mg/kg (1.3±0.2)] compared with control (6.4±0.4) in indomethacin-induced ulcer models. Celecoxib in combination with SC-560 caused significant gastric damage with gastric ulcer score of 4.3±0.4, which was significantly reduced by risperidone (0.5 mg/kg; 1.6±0.2). Risperidone significantly inhibited GAS induced by histamine and pentagastrin but not GAS produced by carbachol. The GMS (mg/g tissue $x10^{-2}$) increased significantly in the 0.1mg/kg (1.1±0.1), 0.3mg/kg (1.3±0.1) and 0.5mg/kg (1.4±0.2) compared with the control (0.6±0.03). There was a dose-dependent increase in the GMCC ($mm^2$) in risperidone-treated groups [0.1mg/kg (121.2±5.0), 0.3mg/kg (129±2.5) and 0.5mg/kg (129±3.8)] relative to the control (103.3±4.2). Malondialdehyde (nmol/L x $10^{-9}$) level was significantly decreased by risperidone [0.1mg/kg (194.0±0.01), 0.3mg/kg (183.0± 0.01) and 0.5mg/kg (106.0± 0.01)] compared to control group (257.0± 0.01). Histology revealed reduced mucosal epithelial and lamina propria damage in risperidone-treated groups compared with control.

Risperidone reduced gastric ulceration via mechanisms related to inhibition of gastric acid secretion mediated through histamine $H_2$ and gastrin receptors. Its anti-ulcer property may also be related to its antioxidant, gastroprotective and cyclooxygenase modulating activities.

**Keywords:** Risperidone, Starvation-induced gastric ulcer, Gastroprotection, Cyclooxygenase modulation

**Word Count:** 500

## DEDICATION

This work is dedicated to God Almighty for His grace and mercies towards me, and to my late father Mazi Jacob Anosike Onwuchekwa, whose desire was the education of his children.

# ACKOWLEDGEMENTS

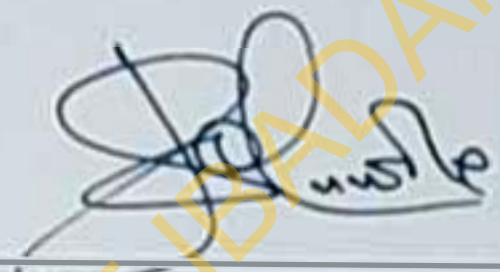
My appreciation goes first to God almighty for His grace towards me and sparing my life till this day. I sincerely thank my able supervisor, Dr Francis Sunday Oluwole for his kindness, encouragement, reading through the scripts and offering useful suggestions. God bless you.

I am also indebted with gratitude to Dr Solomon Umukoro of the Department of Pharmacology and Therapeutics, Dr (Mrs) Adeola Tabitha Salami, Dr Samuel Adetunji Onasanwo, Dr Samuel Babafemi Olaleye and Professor Yunusa Raji of the Department of Physiology for their help and advice during this work. I appreciate the encouragement and co-operation received from the Head of Department, Professor Adesoji Fasanmade and the entire lecturers of the Department of Physiology. I want to thank the entire members of the laboratory of the Department of Physiology for their co-operation and help, most especially Mr Bassey Okon for his assistance in the gastric acid secretory study. I wholeheartedly do appreciate my employer, Usmanu Danfodiyo University, Sokoto, for granting me leave to embark on this study.

My special thanks go to my loving friend and wife Mrs Evelyn Nwakaego Onwuchekwa and my lovely children Ugochi, Chidinma, Ogechi, Tochi and Chigozie for their love. I also acknowledge the help and cooperation of my family members and friends (most especially Ejike Onwuchekwa, Okezie Onwuchekwa and David Nneji). God bless you all in Jesus name.

# CERTIFICATION

I certify that this work was carried out by Mr Chinedu ONWUCHEKWA in the Department of Physiology, University of Ibadan, Ibadan Oyo State, Nigeria.



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| | |
|---|---|
| *i.v.* | Intravenous |
| *i.p.* | Intraperitoneal |
| Kg | Kilogram |
| mg | Milligram |
| $\mu$ | Microgram |
| ml | Millilitre |
| SEM | Standard Error of Mean |
| % | Percentage |
| ATPase | Adenosine Triphosphatase |

# CHAPTER ONE

## 1.0 INTRODUCTION

The term peptic ulcer disease (PUD) is commonly used to refer to ulcerations of the stomach, duodenum, or both, but peptic ulcers can develop in any portion of the gastrointestinal tract that is exposed to acid and pepsin in sufficient concentration and duration. PUD usually occurs in the stomach and proximal duodenum. However, it occurs rarely in the lower oesophagus, the distal duodenum, or the jejunum, as in unopposed hypersecretory states such as the Zollinger- Ellison syndrome, in hiatal hernias (Cameron ulcers), or in ectopic gastric mucosa (e.g., in Meckel's diverticulum). Approximately, 500,000 new cases and 4 million recurrences of peptic ulcers occur in the United States each year (Kurata, 1989). The lifetime prevalence of PUD in the United States is 12% in men and 10% in women. Moreover, an estimated 15,000 deaths per year occurred as a consequence of complicated PUD. The financial impact of PUD is substantial with an estimated burden on direct and indirect health care costs of $10 billion per year in the United States (Valle, 2008).

PUD includes both gastric and duodenal ulcers. Ulcers are defined as breaks in the mucosal surface >5 mm in size with depth to the submucosa. Duodenal ulcers (DUs) and gastric ulcers (GUs) share many common features in terms of pathogenesis, diagnosis, and treatment, but several factors distinguish them from one another. Helicobacter pylori (H. pylori) and non-steroid anti-inflammatory drugs (NSAIDs) induced injury account for the majority of DUs.

Many acid secretory abnormalities have been described in DU patients. DUs are estimated to occur in 6-15% of the Western population. The incidence of DUs declined steadily from 1960 to 1980 and has remained stable since then (Valle, 2008). The reason for the reduction in the frequency of DUs is likely related to the decreasing frequency of H. pylori. DUs occur most often in the first portion of duodenum (>95%), with 90% of them located within 3 cm of the pylorus. They are usually greater or equal to one (≥1) cm in diameter but can occasionally reach 3-6 cm (giant ulcers). Ulcers are sharply demarcated with their depth at times reaching the museularis propria. The base of the ulcer often consists of a zone of cosinophilic necrosis with surrounding fibrosis. Malignant DUs are extremely rare.

Gastric ulcer is a multifactorial etiological disease. Several factors which play a significant role in gastric ulcerogenesis include stress, trauma, sepsis, hemorrhagic shock, burns, *Helicobacter pylori*, steroidal and non-steroidal drugs (Feldman et *al.*, 2002; Hoodenveif et *al.*, 2006). Regardless of great advances in the field of medical science and understanding of the peptic ulcer illness, gastric ulcers aetiology is still not completely understood. The most important factor responsible for the genesis of gastric ulcers is the imbalance between the defensive factors, such as secretion of mucus and bicarbonates, and offensive factors, such as increased secretion of acid and pepsin (Ramakrishnan and Salinas, 2007). Thus, gastric ulcer is a benign lesion of the gastric mucosa, which occurs at the site where the mucosal epithelium is continuously exposed to acid and pepsin (Andrade *et al.*, 2007). GUs tends to occur later in life than duodenal lesions, with a peak incidence reported in the sixth decade. More than half of GUs occurs in men. GUs is less common than DUs and has a higher likelihood than DUs of being silent and presenting only after a complication develops. In

contrast to DUs, GUs can represent a malignancy. Benign GUs are most often found distal to the junction between the antrum and the acid secretory mucosa. Benign GU is quite rare in the gastric fundus and is histologically similar to DUs. The majority of GUs can be attributed to either H. pylori or NSAID-induced mucosal damage. GUs that occur in the prepyloric area or those in the body associated with a DUs or a duodenal scar are similar in pathogenesis to DUs. Under normal conditions, a physiologic balance exists between peptic acid secretion and gastroduodenal mucosal defence. Mucosal injury (peptic ulcer) occurs when the balance between the aggressive factors and the defensive mechanisms is disrupted.

Aggressive factors include abnormalities in gastric acid secretion and H. pylori infection. Increased gastric parietal cell mass, increased maximal, basal, daytime, and nocturnal acid output (Blair *et al.*, 1987; Merki *et al.*, 1988). Other aggressive factors for DU are increased duration of meal-stimulated acid secretion and fasting serum gastrin levels (El-Omar *et al.*, 1995) and decreased bicarbonate production by the proximal duodenum. On the other hand increased serum levels of pepsinogen (Samlo *et al.*, 1986), increased duodenogastric reflux (Fisher and Cohen, 1973), and decreased gastric parietal cell mass as well as maximal acid output are aggressive factors for GU. NSAIDs represent a group of the most commonly used medications and asymptomatic ulcerations have been documented endoscopically in 15% to 45% of patients on chronic NSAID therapy (Laine, 2001). However, 1% to 4% of patients receiving NSAIDs for one year will experience serious gastrointestinal complications (Laine, 2001). NSAID-induced injury could be either due to inhibition of the cyclooxygenase enzyme, inhibiting prostaglandin synthesis which impairs mucosal defence and repair and facilitates mucosal injury or direct local mucosal toxic processes.

The mechanism responsible for increased ulcer diathesis in smokers is unknown. Theories have included altered gastric emptying, increased gastric acidity, increased maximal acid output, decreased proximal duodenal bicarbonate production increased risk for H. pylori infection and cigarette-induced generation of noxious mueosal free radicals ((Ainsworth *et al.*, 1993; Bateson, 1993).

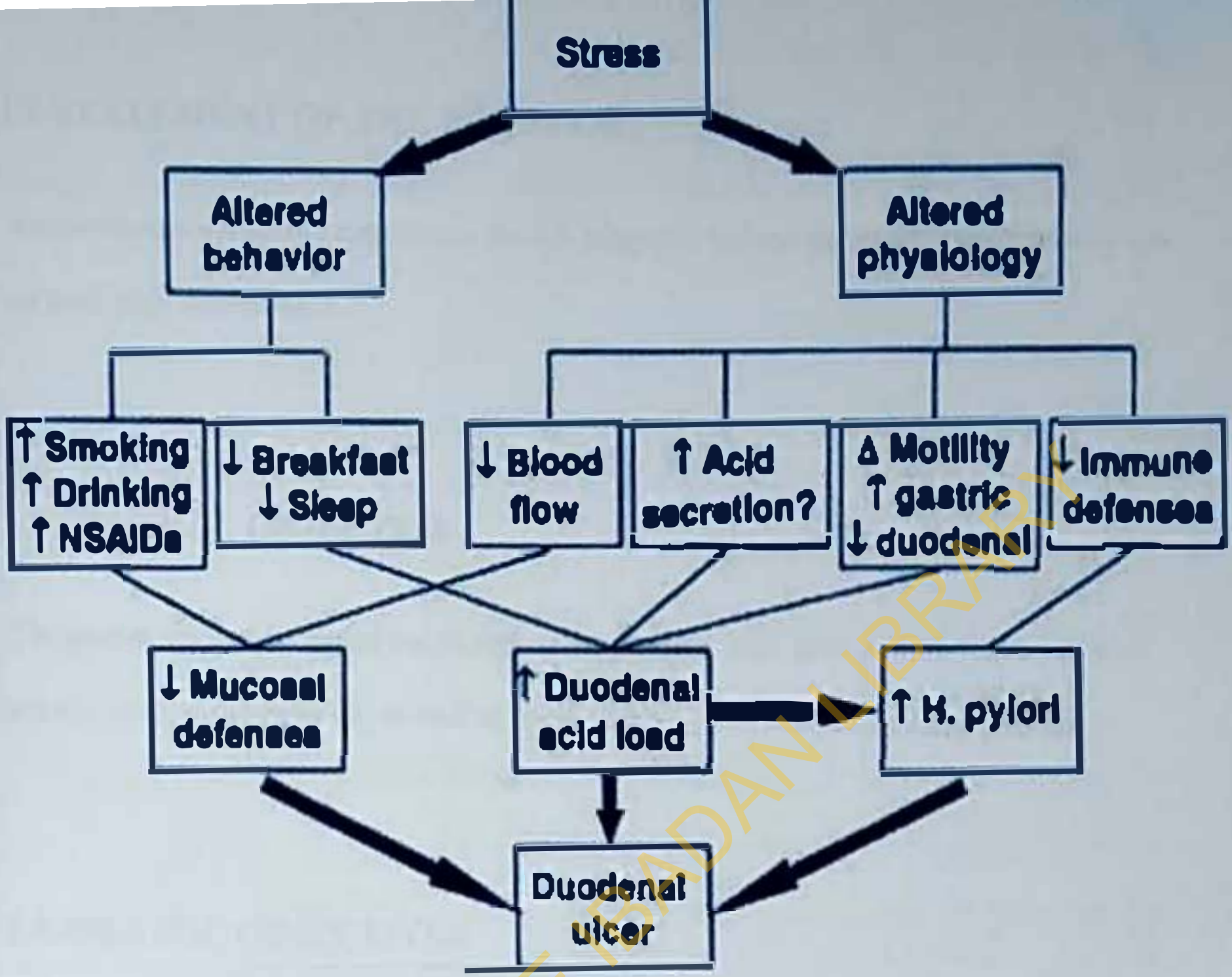
Proposed genetic markers for ulcer disease include blood group O antigen, the lack of secretion of blood group antigens in the saliva, and the presence of certain human leukocyte antigen subtypes (Rotter, 1983). No study has established a convincing link between diet and PUD. Ulcer patients often describe dyspepsia associated with the ingestion of certain foods, especially spicy foods, but the evidence that such foods cause ulceration is virtually lacking. Coffee, tea, and colas are potent gastric acid secretagogues (McArthur, Hogan and Isenberg, 1982), but epidemiologic studies have not established an association between these beverages and PUD. Rarely, PUD results from disorders that cause the stomach to secrete gastric acid in quantities so large that they overwhelm the normal epithelial defense mechanisms. These disorders include gastrinoma (Zollinger- Ellison syndrome) or multiple endocrine neoplasia, antral G cell hyperplasia, systemic mastocytosis, and basophilic leukemias.

A number of reports have suggested that emotional stress might cause or exacerbate peptic ulceration (Walker and Feldman, 1992). Some antidepressant drugs reported to have antiulcer activity are doxepin (Shrivastava *et al.*, 1985; Hamid and Taghi, 1997), fluvoxamine (Dursun *et al.*, 2009), trimipramine (Guldahl *et al.*, 1977; Anderson *et al.*, 1984) and monoamine oxidase type B (MAO-B) inhibitors (de Abajo *et al.*, 1999). While, anti-psychotic drugs with anti-ulcer activity, are perospirone (Ishida- Tokuda *et al.*, 1996) and risperidone

(Saxena and Singh, 2011). Steroid and non-steroidal drugs, cigarettes, alcohol usage, trauma, sepsis, shock, Helicobacter pylori, and stress have been shown to contribute to gastric ulcer formation (Mózsik and Jávor, 1988; Davies *et al.*, 1994; Ding *et al.*, 1998; Hoodcrwerf and Pasricha, 2006).

Stress is one of the aggressive factors in peptic ulcer formation (Figure 1) and underlies many other diseases apart from ulcers, for example, depression. Stress is one of the most commonly used methods to produce ulcer models (Brzozowski *et al.*, 2008; Kwiecień *et al.*, 2007). Depression, accompanied by psychotic and somatic symptoms, is present in most patients with gastrointestinal system (GIS) ulcers (Guldahl, 1977). An increased vulnerability to depression (Pare, 1989) and anxiety (Glavin, 1993) in experimental animals is paralleled with ulcer development and the same holds true for humans (Sjodin *et al.*, 1985; Feldman *et al.*, 1986). Moreover, antidepressants (Rics *et al.*, 1984; Mangla *et al.* 1982) and anxiolytics (Shrivastava and Siegel, 1984; Haggerty and Drossman, 1985) can significantly reduce stress ulcer formation, perhaps to a greater extent than that seen with traditional therapies such as cimetidine and antacids (Shrivastava et *al.*, 1985). Gastric side effects of selective serotonin reuptake inhibitor (SSRI) drugs have been reported (Lewis *et al.*, 2008). The combined usage of SSRI drugs and indomethacin has been reported to cause gastrointestinal bleeding (de Abajo *et al.*, 1999). However, Glavin *et al.*(1998) reported that several novel arylpiperazine serotonin 1A receptor ($5HT_1$-A) agonists, developed as anxiolytics, have antisecretory and gastroprotective effects in rats. In addition, $5HT_1$-A antagonists increase the potency of serotonin-related contractions in stomach tissue (Burka *et al.*, 1989), while the $5HT_1$-A agonist buspiron decreases stomach and intestinal distension (Tack, 1999). In the light of this

literature, it can be hypothesized that the antiulcer effect of risperidone may be related to the stimulation of $5HT_1$- A receptors, but further detailed studies are required to clarify this point. Thus, gastric ulcer is caused by imbalance between the gastroduodenal mucosal defensive factors such as bicarbonate, mucus and aggressive factors such as acid and pepsin (Sostres and Lanas, 2011). Many of the anti-ulcer drugs in use have been found to have adverse effects recurrent infection after a few weeks (Chan and Leung, 2002). However, the goals of treating gastric ulcer include relief of pain, healing of the ulcer and prevention of its recurrence (Sostres and Lanas, 2011). The successful treatment of gastric lesion depends on augmentation of the defensive factors of the gastric mucosa and blockage of acid secretion (Borelli and Izzo, 2000).




↑ Drinking = ↑ Alcoholic Drinking

↑ H. Pylori = ↑ Helicobacter pylori

Figure 1 Diagram of how stress induces peptic ulcer (Brzozowski *et al.*, 2008)

## 1.1 STATEMENT OF THE PROBLEM

Antipsychotics espccially risperidone though suspected to have gastroprotection activity has not been fully documented.

## 1.2 GENERAL OBJECTIVE

The present study was carried out to explore the possible anti- gastric ulcer activity of the antipsychotic, risperidone (R) as well as its mechanism(s) of action in male Wister rats.

## 1.3 SPECIFIC OBJECTIVES

The work was divided into six major studies;

1. To investigate the acclaimed anti-ulcer effect of risperidone using different ulcer models; Water immersion-restraint stress (WIRS) - , Indomethacin- and Starvation- induced gastric ulcer.

2. To determine the anti-secretory effect of risperidone on basal and maximal secretion using three agonists namely histamine, pentagastrin (gastrin) and carbachol ( an analogue of acetylcholine).

3. To investigate the gastroprotective activity of risperidone by determining its effect on gastric mucus secretion and gastric mucus cell counts.

4. To determine the effect of risperidone on indomethacin, cyclooxygenase 1 inhibitor (SC - 560) and cyclooxgenase 2 inhibitor (celecoxib) induced gastric ulceration.

5. To determine the antioxidant status of risperidone on treated animals by measuring malondialdehyde (MDA) concentration.

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

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

## 2.1 STRUCTURE AND FUNCTION OF 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 below the diaphragm, is a distensible sac-like structure with strong, muscular walls (figure 2). 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 (Ganong, 2003). 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 (Ganong, 2003). 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 meals. 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 (Ganong, 2003).

## 2.2 HISTORY OF GASTRIC ACID STUDY

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 favoured 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

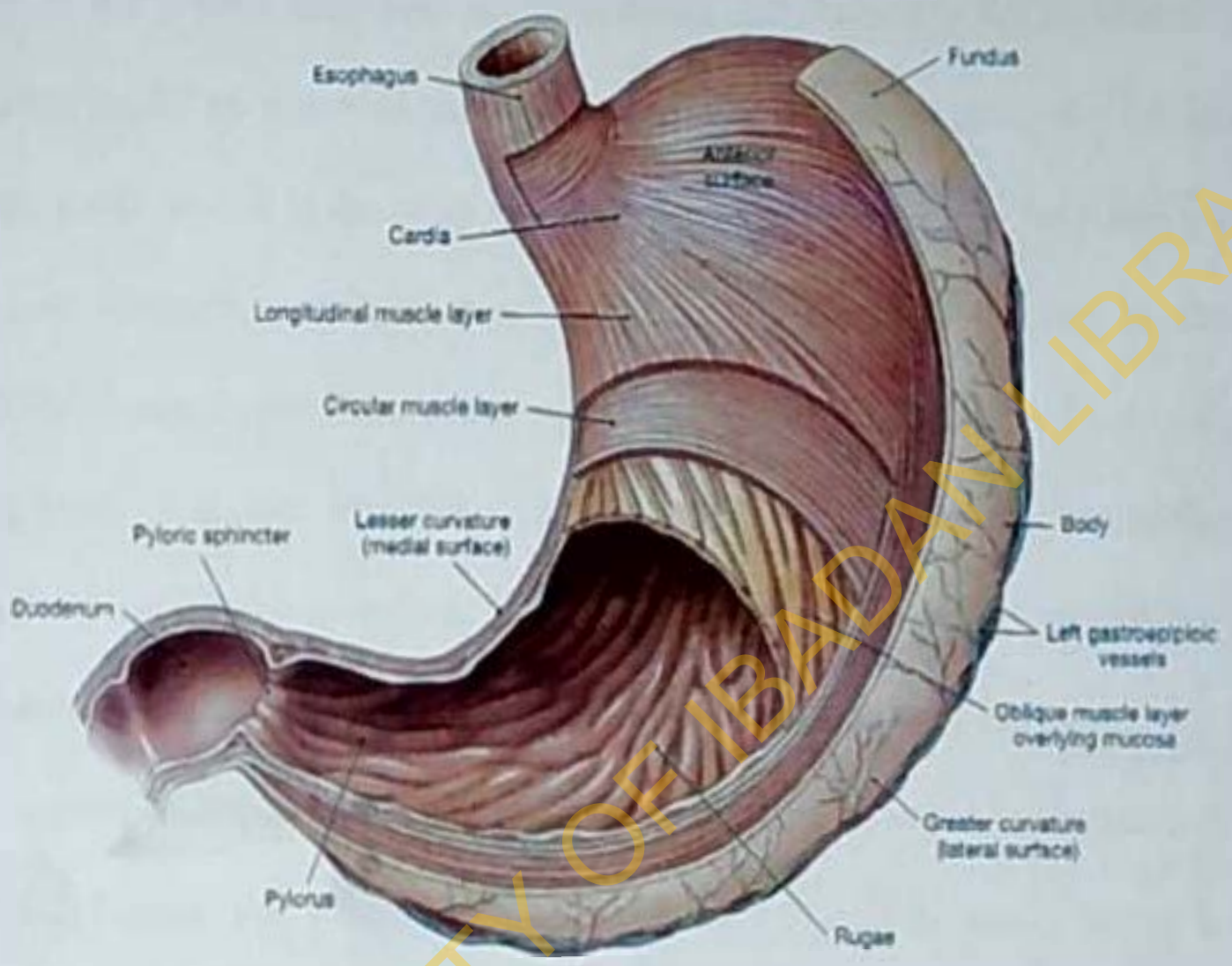



Figure 2 Diagram showing the anatomical structure of the stomach (Hersey and Sach. 1995)

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. 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 fibres travelled 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 oesophageal 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 oesophageal fistula in order to eliminate stimulation of the oral cavity. As such, gastric juice is obtained. However, 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 these methods to describe the membrane transport across the gastric mucosa and several other chemical events. 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 and sham feeding.

### 2.3.1 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.

### 2.3.2 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.

### 2.3.3 Continuous perfusion of the stomach

This method was devised by Ghosh 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 intraperitoneal injection of urethane (25%) at a 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 oesophageal cannula of the Watson Marlow's flow meter or the modified Langerdoff's apparatus is inserted through the mouth of the animal. The perfusing fluid,

which is nonnal saline, is then run through the oesophageal 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.

### 2.3.4 Gastric pouch preparation

This method allows for direct investigation into the functions of the stomach. The first gastric pouch was developed by Pavlov (1910). Other scientists had since modified the Pavlov's pouches (Heidehain, 1987; Katch, 1912; Ivy, 1926). 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.

### 2.3.5 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 oesophageal 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).

## 2.4 MAJOR COMPONENTS OF GASTRIC JUICE

### 2.4.1 Role of Parietal Cells in Gastric Acid Secretion

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). 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:

1. $H_2$ receptors that respond to histamine from the enterochromaffin-like cells
2. $M_3$ 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

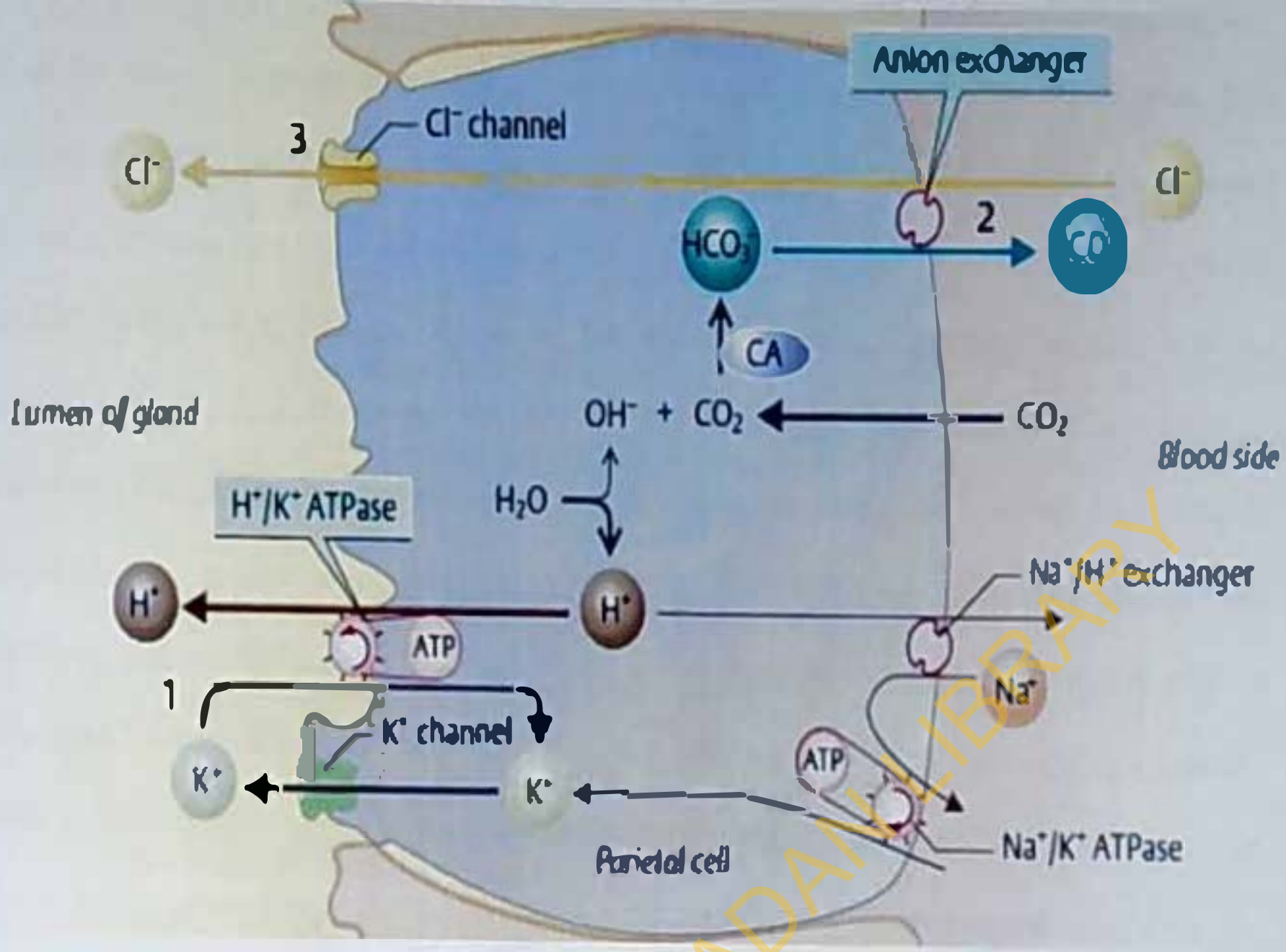
Early investigations defined most of our knowledge about gastric chloride secretion but are derived solely from in vivo (steady state) experiments or in vitro preparations of isolated

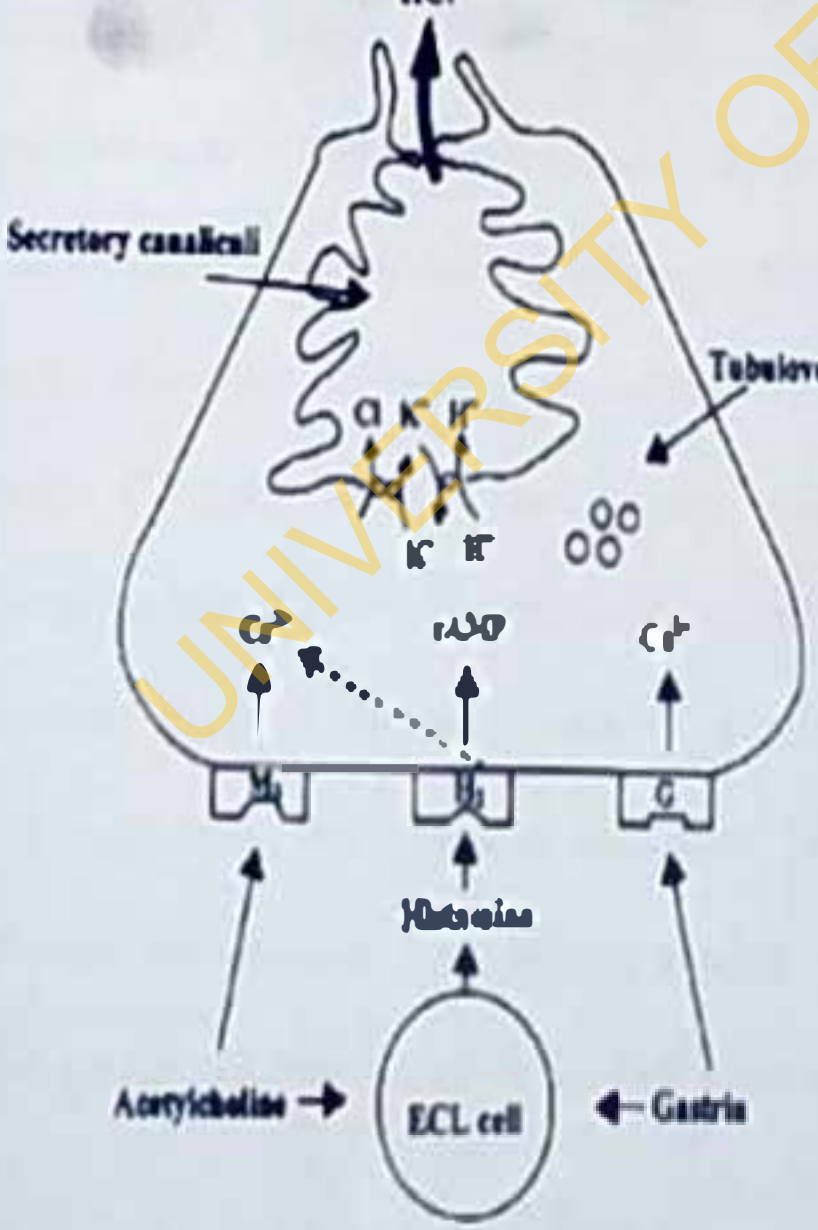
amphibian mucosa. More recent information from mammalian systems have identified baso-lateral $Na^+$-$K^+$-$Cl^-$ co-transporter NKCC1 and $Cl^-$-$HCO_3^-$ exchanger, AE2 as potential routes for chloride uptake by gastric epithelial cells and has identified a CLC-2 as a pH sensitive $Cl^-$ channel that 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. Figure 3 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. 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 (Ganong, 2003).

## 2.5 MECHANISM OF HCL SECRETION BY PARIETAL CELL

The most recent theory of the mechanism of gastric acid secretion is the $H^+$-$K^+$-ATPase theory which is as follows:

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. Chloride ions are actively transported from the cytoplasm of the parietal cell into the lumen of the canaliculus and sodium ions are actively transported



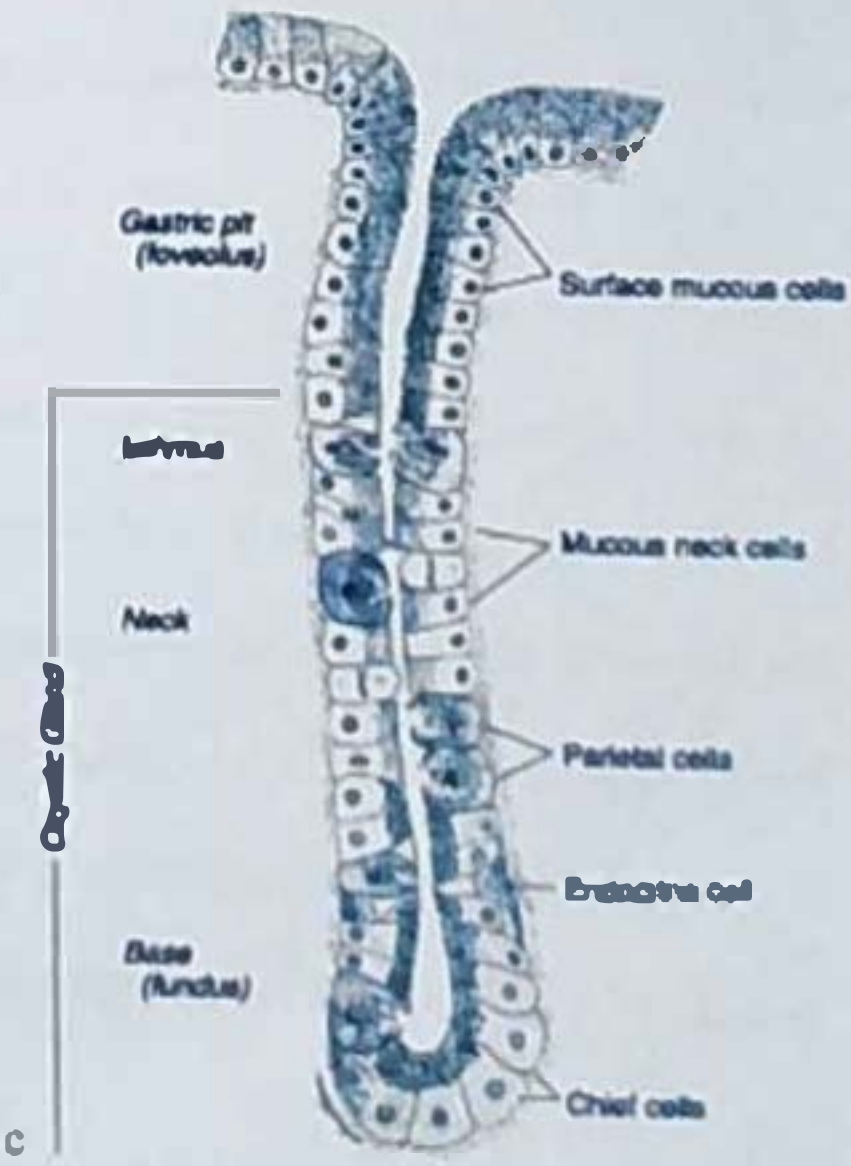








Figure 3 Composite diagrams of a parietal cell (Guyton and Hall, 2000)

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. Water dissociates into $H^+$ and $OH^-$ in the cell cytoplasm (figure 4). 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 are replaced by hydrogen ions in the canaliculus giving a strong solution of hydrochloric acid which is secreted into the lumen of the oxyntic gland (Ganong, 2006). 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 (Ganong, 2006). 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 (Ganong, 2003). 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 reaches the stomach is 160 mM per

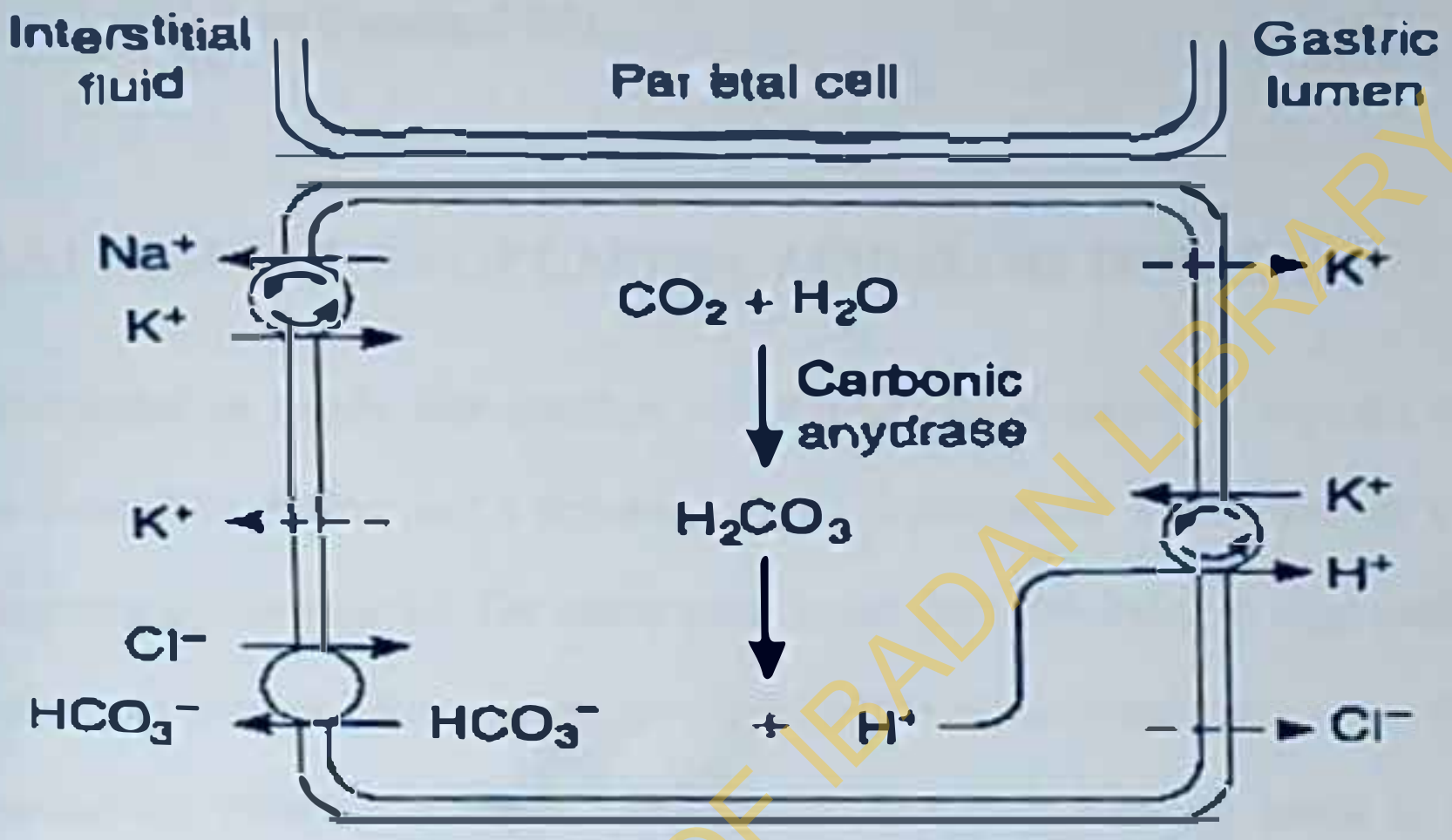



Figure 4 Secretions of hydrochloric acid by parietal cells in the stomach (Ganong, 2007).

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 (Ganong, 2003).

## 2.5.1 REGULATION OF GASTRIC ACID SECRETION (GAS)

Stimulation of gastric acid secretion was divided into a central or cephalic phase, as envisioned by Pavlov, and a peripheral phase, as determined by injection of a putative regulator of acid secretion. The central phase results from stimulation of vagal outflow from the central nervous system and post-ganglionic release of neurotransmitters within the gastric epithelium, while the peripheral phase is from exocytotic events in gastric or intestinal endocrine cells. The central post-ganglionic pathway converges on either the gastric endocrine cells or the parietal cell or both. Therefore, a definition of neurally mediated regulation is alteration in secretion due to a direct effect of substances released from gastric nerves; peripheral regulation derives from alteration in secretion due to substances released from endocrine cells (Pavlov, 1910; Duke *et al.*, 1965; Tache and Yang, 1990). At the turn of the century, Edkins discovered gastrin (Edkins, 1906). Loewi discovered acetylcholine (Loewi, 1921) and while histamine was by Dale (Barger and Dale, 1911). So by the 1920s, many of the peripheral secretagogues that are cited today had been described although it took half a century for the idea of gastrin to be accepted. However, the gastrinologists discounted any role for histamine.

## 2.5.1.1 PHASES OF GASTRIC ACID SECRETION

Several years ago, regulation of gastric acid was divided into three phases.

**a. The cephalic phase**

Thirty percent (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 for food. Vagal impulses cause the release of acetylcholine in the body of the stomach. Acetycholine both directly increase gastric acid secretion and also stimulate bistamine release.

**b. The gastric phase**

Sixty percent (60%) of the acid secreted is stimulated by the distension of the stomach with food and proteins produced from 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 mechanism in the control of gastric acid secretion.

**c. The intestinal phase**

The remaining 10% of acid is secreted when chyme enters the small intestine, and is stimulated by distension of the small intestine.

Current concept has broadly divided these modes of regulation into two namely; central and peripheral. Also gastric acid secretion (GAS) whether in response to peripheral and/or central administration of chemical or electrical stimuli can be differentiated by vagotomy.

## 2.5.2 CELLULAR BASIS OF REGULATION OF ACID SECRETION

The first 25 years of the twentieth century defined the major mechanisms of stimulation of acid secretion. Gastrin, a hormone released from the antrum (Edkins, 1906), and acetylcholine, released from the vagus (Loewi, 1921), were early upon the scene, dividing regulation of acid secretion into a peripheral and central phase, respectively. Although it was recognized that histamine was a potent stimulus of acid secretion (Popielski *et al.*, 1920), histamine remained for many years a controversial actor on this stage.

Therapeutic regulation of acid secretion until 1973 depended either on surgical intervention or on the use of vagal blockade by extract of belladonna or atropine. In early1970s, the introduction of the first $H_2$-receptor antagonist, cimetidine, not only changed medical therapy but also changed our understanding of gastric regulatory physiology (Black *et al.*, 1972). This $H_2$-receptor antagonist not only blocked histamine-induced acid secretion, but also gastrin induced acid secretion and blocked much, but not all, of the vagally-mediated acid secretion (Black *et al.*, 1972). Since the histamine molecule bears no resemblance to either gastrin or acetylcholine, evidently the release of histamine is a major regulatory event in the stimulation of acid secretion. In vivo experiments suggested that an histamine-containing cell in the gastric mucosa, the enterochromaffin-like (ECL) cell, was the cell stimulated by gastrin or acetylcholine to release histamine. This histamine release mediated all or most of the stimulation of gastric acid secretion (Hakanson *et al.*, 1986). This cell type, not the mast cell, was in the right location and showed the right responses for an intermediary in secretory stimulation. Gastrin release from the antral G cell accounts for most of the stimulation of histamine release from the ECL cell in vivo, and therefore, the G cell plays a vital role in

stimulation of acid secretion. The somatostatin-containing D cell is located both in fundus and antrum. and somatostatin inhibits both G and ECL cell function. Regulation of acid secretion by gastric endocrine cells involves, therefore, positive and negative interactions between a triumvirate, G, ECL and D cells (Sachs *et al*., 1994).

**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 (cAMP). 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 conditions including peptic ulcers and gastro-oesophageal reflux disease. Examples include cimetidine, 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 acetylcholine 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 histidine decarboxylase- knockout (HDC-KO) mice in which histamine release from ECL cells was 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 wild type (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. The acid

secretagogue effect of the muscarinic agent carbachol and therefore endogenous muscarinic activity is mainly exerted directly on the parietal cell in the rat. Thus, the muscarinic agent carbachol may be stimulating acid secretion and $H^+$-$K^+$ ATPase mRNA in vivo through a direct effect on the parietal cell, not dependent on the release of ECL cell histamine (Sandvik *et al*., 1988).

## c. Gastrin

Gastrin released from G cells has been reported to be directly stimulated by acetylcholine via muscarinic receptors, $M_3$ and $M_3$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). The 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). GAS has been shown to be controlled by specific lateral hypothalamic (LHA) neurons. The paraventricular nucleus (PVN) has now been found to also affect GAS. GAS was produced more copiously and more quickly by rostral PVN lesion than by lesion of the ventromedial (VMH) or dorsomedial (DMH) nucleus, and nearly as much by caudal PVN lesion. Results from studies indicate that the PVN may be an additional central site from which GAS is affected. Strong parasympathetic stimulation to the stomach leads to increased gastric acid secretion (Shiraishi and Simpson, 1987).

**d. Physiological functions of Gastrin and Histamine**

Similarly, gastrin increases venous histamine output from the canine stomach (Gerber & Payne, 1992). Also, gastrin has been reported to release histamine from small-cell enriched fractions of gastric fundic mucosal cells from rat, rabbit and dog (Roche *et al.*, 1991a, b; Chuang *et al.*, 1992; Prinz *et al.*, 1993; Sandor *et al.*, 1996). It is now clear that a neuroendocrine cell, the enterochromaffin-like (ECL) cells, synthesizes stores and releases histamine upon gastrin stimulation (Roche *et al.*, 1991a, b; Prinz *et al.*, 1993; Sandor *et al.*, 1996). It is also a target for gastrin in stimulating ECL cell growth in animals and in man (Borch *et al.*, 1985; Loé Nioth *et al.*, 1990; Bordi *et al.*, 1995). Shankley *et al.*, (1992) reported that pentagastrin produced a concentration-effect curve in the presence of histamine $H_2$-receptor blockade, presumably due to a direct action of pentagastrin on the oxyntic cell, which would be independent of histamine release. They also reported that the action of pentagastrin, under control conditions, is due to both an indirect, histamine-mediated action and a direct action on the oxyntic cell. Similarly, gastrin increases venous histamine output from the canine stomach (Gerber & Payne, 1992). Also, gastrin has been reported to release histamine from small-cell enriched fractions of gastric fundic mucosal cells from rat, rabbit and dog (Roche *et al.*, 1991a, b; Chuang *et al.*, 1992; Prinz *et al.*, 1993; Sandor *et al.*, 1996).

## 2.5.3 CENTRAL PEPTIDERGIC CONTROL OF GASTRIC ACID SECRETION

Recent advances in neurosciences, have provided new insights into gut physiology and the nature of brain-gut interaction. As well as demonstrating specific roles for many peptides in the brain-gut axis, these developments have yielded valuable insights into aspects of gut

physiology (Table 1). The connection between emotional perturbation and disorders of the stomach must have been apparent even to the most primitive of human cultures. Early clinical and animal experimental observations provided evidence of a functional link between the brain and the gut. The increase in biochemical and immunohistochemical techniques that occurred during the 1960s led to the identification of a large number of peptide neurotransmitters in the brain. The demonstration that substance P, vasoactive intestinal peptide, and somatostatin were present in both the brain and the gut raised questions about the nature of brain-gut interaction (Krulich *et al.*, 1968; Chang and Leeman, 1970; Fuxe *et al.*, 1977). The subsequent identification by Dockray in 1976 of high concentrations of cholecystokinin (CCK) in the brain of rats, pigs, and dogs was of enormous importance (Dockray, 1976). Here was one of the preeminent gut peptides, with a well established hormonal action, unexpectedly identified in high concentrations in the brain where it functioned as a neurotransmitter. In many instances peptide and non-peptide neurotransmitters are co-localised in the same neural terminals where they may interact functionally. Non-peptide neurotransmitters undoubtedly play a part in central regulation of gastric secretion. Indeed, many neuropeptides were shown to have powerful effects on gastric acid secretion. The preponderance of inhibitory over stimulatory peptides is not surprising as the efficiency of any secretory system depends largely on its inhibitory mechanisms.

### a. Thyrotropin Releasing Hormone

Thyrotropin releasing hormone (TRH) was the first brain peptide shown to affect gut function (Smith *et al.*, 1977). When TRH was first identified in 1970, it was assumed that it served a

**Table 1 Peptides that affect gastric acid secretion**

| Inhibitors | Stimulators |
|---|---|
| CRF | TRH |
| β endorphin | Somatostatin |
| Bombesin | NPY |
| Neurotensin | Galanin |
| Calcitonin | PYY |
| CGRP | |
| Interleukin 1 | |

(Scoto and Parenti, 1993; Yang and Tache, 1995)

specific function in the hypothalamo-hypophyscal axis. Immunohistochemical localisation studies showed, however, that TRH was also found in other extra-hypothalamic sites in areas known to be important for integration of visceral function, with high concentrations in the vagal nuclei in the brainstem. In 1977, Smith and colleagues reported that intracerebrovcntricular injection of TRH caused an increase in colonic motor activity in rabbits (Smith *et al.*, 1977).This was the first report of a central effect of a neuropeptide on gut function. Tache subsequently reported that intracisternal TRH has a potent stimulatory effect on gastric acid secretion in rats that was completely independent of its hypophysiotropic action (Tache *et al.*, 1985). Central administration of TRH also increases gastric emptying and gastric mucosal blood flow in rats (Tache *et al.*, 1989). A considerable amount of convergent evidence, including localisation, immunoneutralisation and analogue studies, supports the hypothesis that TRH plays a physiological role in central regulation of gastric acid secretion. The highest concentrations of TRH receptors are found in the dorsal vagal nuclei (Palkovits *et al.*, 1986), the final common pathway for vagal outflow from the brain to the stomach. Many different higher centres exert an influence on gastric acid secretion and it has been proposed that these higher signals converge through this TRH mediated pathway.

**b. Bombesin**

Evidence is accumulating that bombesin also has a significant role in central control of acid secretion (Pappas *et al.*, 1985). Central injection of bombesin potently inhibits acid secretion in response to a variety of central and peripheral stimulants, the opposite of its well

established peripheral stimulatory effect on acid secretion. The central inhibitory action of bombesin is not affected by vagotomy, but is abolished by cervical transection and $\alpha_2$ adrenergic blockade, showing that its central effect is mediated through the sympathetic nervous system (Tache *et al.*, 1986). Indeed, many of the peptide effects described have been shown to be mediated by non-vagal pathways.

### c. Corticotropin Releasing Factor

The physiological response to stress involves a complex array of adjustments in cardiorespiratory, gastrointestinal and endocrine function that equips the subject to deal with stress. Although the peripheral manifestations of stress response are well described, corticotrophin releasing factor (CRF) has been identified as an important mediator of the stress response. Even though CRF was first identified in the hypothalamo-hypophyeal axis, it is also present in extra-hypothalamic sites in the CNS. Studies show that it elicits the full spectrum of cardiovascular, metabolic, endocrine, gastrointestinal and behavioural effects associated with stress (Tache *et al.*, 1988). The potent inhibitory effect of intracerebroventricular CRF on gastric acid secretion was first described by Tache and associates (Lenz *et al.*, 1988). This inhibitory expetimental action is compatible with the findings that stress ulceration in animal models and in stressed patients is associated with decreased rather than increased acid secretion. CRF also causes retardation of gastric emptying, and small bowel transit, and a pronounced increase in colonic motility ((Tache *et al.*, 1988). These effects are produced by interaction with a number of other neurotransmitters including opiates and are probably, at least in part, vagally transmitted (Tache *et al.*, 1988).

### d. Opioids

A physiological role for opioids in modulating the gastric response to stress has been demonstrated by studies using opioid antagonists. Central administration of opioids inhibits acid secretion by a vagal mechanism. These agents also inhibit the formation of stress-induced gastric ulcers suggesting that opioids protect against stress-induced alterations in gastric function (Scolo and Parenti, 1993).

### e. Neurotensin

Neurotensin has also been implicated as a mediator of the stress response. Centrally administered neurotensin decreases gastric acid secretion and has a protective effect on stress-induced gastropathy in a number of animal models (Zhang *et al.*, 1989).The central site of this effect is probably in the limbic system, and entails interaction with adrenergic and dopaminergic pathways (Zhang *et al.*, 1989; Pappas *et al.*, 1987). The peripheral effect is produced by changes in peripheral prostaglandin production and gastric mucosal blood flow.

### f. Calcitonin and Calcitonin Gene Related Peptide (CGRP)

Calcitonin and calcitonin gene related peptide (CGRP) may also be involved in gastric mucosal protection (Morley *et al.*, 1981; Rache, 1992; Gray *et al.*, 1994.), although they seem to act through different mechanisms. Central calcitonin inhibits the development of stress-induced ulcers (Morley *et al.*, 1981). Most evidence points to a peripheral role for CGRP in gastric cytoprotection, however, some studies suggest that it may also have a central effect, which seems to be produced by modulation of central parasympathetic outflow resulting in increased mucosal blood flow.

**g. Interleukin 1**

Several studies have demonstrated interaction between gut peptides and mediators of immune responsiveness within the gastrointestinal tract. A recent report showed that intracerebroventricular interleukin 1 inhibits acid secretion in rats, providing evidence that immunological mediators may also affect gut function through central mechanisms (Tache and Saperas, 1992). Interleukin 1 also inhibits gastric emptying and small intestinal motility, and stimulates colonic motility through prostaglandin and CRF-mediated pathways.

**h. Neuropeptide Y**

Neuropeptide Y (NPY), a member of the pancreatic polypeptide family, is the most potent known stimulant of feeding and is found in higher concentrations within the CNS than any other neuropeptides. Its potency as an exigenic agent and the preservation of its effects across a range of species suggest that NPY plays an important part in the initiation of feeding behaviour. Recent observations of the effect of central administration of NPY in dogs show that NPY cannot only initiate feeding behaviour but can also trigger a range of gut secretory and motor effects, which normally occur at the onset of feeding (Geoghegan *et al.*, 1993). Central administration of NPY increased not only gastric acid (by a vagal pathway), but also pancreatic and biliary secretion and converted the fasting pattern of cyclical myoelectric activity in the stomach and small intestine to the pattern seen after feeding (Farouk *et al.*, 1992; Thompson *et al.*, 1993). Administration of an NPY antagonist to sham feeding dogs with oesophageal fistula prevented the cephalic phase gastric acid response, proving that the effect of NPY on gastric acid secretion is a physiological one (Lee *et al.*, 1994). Galanin,

another initiator of feeding behaviour, has a similar though less potent central effect on acid secretion (Geoghegan *et al.*, 1990). These studies suggest that NPY as well as being involved in the initiation of feeding behaviour might also be responsible for triggering the full range of secretory and motor responses that prepare the gut for ingested food. The single peptide may be responsible for coordinating a broad range of functions that serve a common purpose. The concept that the same peptide that is responsible for activating feeding also triggers an appropriate preparatory secretory response is attractive because it suggests the sort of simple economy that characterises many biological systems. CRF, in similar fashion, seems to be responsible for coordinating an integrated response to stress. These findings suggest that peptide neurotransmitters play important integrative function within the CNS, providing templates for coordinated homeostatic and visceral responses to certain specific situations.

## 2.6 PERIPHERAL REGULATION OF GASTRIC ACID SECRETION

This is initiated by the release of gastrin from the G cell. Gastrin then stimulates the cholecystokinin-B receptor on the enterochromaffin-like cell beginning a calcium signalling cascade. An exocytotic release of histamine follows with concomitant activation of a $Cl^-$ current. The released histamine begins the $H_2$-receptor-mediated sequence of events in the parietal cell, which results in activation of the gastric $H^+/K^+$-ATPase. This enzyme is the final common pathway of acid secretion (Sachs *et al.*, 1994). The regulation of gastric acid secretion is achieved in the periphery by interplay between three major gastric endocrine cells: the enterochromaffin-like (ECL) cell, the gastrin or G cell and the somatostatin or D cell. , Regulation of these cells is via stimulatory or inhibitory paracrine, endocrine, and

neural pathways. Upregulation of ECL function is determined by activation of CCK-B receptors, by gastrin, and by activation of beta-adrenergic receptors, as well as by acetylcholine in some (10-29%) of the cells. Gastrin and acetylcholine produce typical biphasic calcium signals. Inhibition of ECL cell histamine release and calcium signalling is produced by somatostatin acting at a type 2 receptor, histamine acting at a histamine-3 receptor, and by peptide PYY. Stimulation of ECL cells results in activation of chloride channels, and there is evidence that voltage-dependent calcium channels, along with the receptor-operated calcium channels, are also responsible for elevation of intracellular concentration of calcium [Ca]. Depolarization-activated $K^+$ channels presumably restore the potential after depolarization by activation of the chloride channel. The D cell is activated by either gastrin or CCK and appears to be inhibited by acetylcholine and somatostatin. The G cell is activated by acetylcholine and gastrin-releasing peptide (GRP) and is inhibited by somatostatin. The functional integration of these three cell types is the primary determinant of the degree of stimulation of the parietal cell (Sachs *et al.*, 1997).

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 gastrinstimulate 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 production of gastric acid. The production of gastric acid in the stomach is tightly regulated by positive regulators and negative feedback mechanisms. Four types of cells are involved in this process: parietal cells, G cells (Buchan, 1991), D cells and enterochromaffin-

ike 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 (GRP) (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 too, by stimulating the release of histamine (Prinz, 1994). 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).

## 2.7 ENDOCRINE CELLS

There are at least three endocrine cells that play a major role in regulation of acid or pepsinogen secretion;

- Enterochromaffin-like cell (ECL)
- Gastrin or G cell and
- Somatostatin or D cell.

The ECL cells produce and store histamine (Hakanson *et al.*, 1986). This biogenic amine is stored in vesicles to give a total content of 2.8 to 4.3 pg/cell of histamine, which is a relatively low amount compared to mast cells (12 to 20 pg/cell). As for other gastric endocrine cells, this is a small, about 10 um diameter, cell found at mostly towards the base of the fundic gastric gland. It contains acidic vacuoles with an eccentric electron dense spot (Hakanson *et*

al., 1971). High doses of omeprazole resulted in ECL cell hyperplasia and carcinoid formation in rats (Larsson *et al.*, 1986; Tielemans *et al.*, 1990; Ryberg *et al.*, 1990; Lee *et al.*, 1992).

The ECL cell is found mainly in the fundic region of the stomach, the G cell in the antral gland and the D cell in both the antral and fundic region. The fundic and antral D cells may differ in some of their receptor properties, given that the antral D cell communicates with the antral gland lumen and is juxtaposed to the G cell and that the fundic D cell does not communicate with the gland lumen and is in the vicinity of the ECL cell (Sachs *et al.*,1997; Forssmann *et al.*,1969).

### 2.7.1 STIMULATION OF ECL CELLS

**a. Peripheral Stimulation**

The major stimulatory ligand for histamine release from these cells is gastrin. Addition of gastrin to a perfusate results in a characteristic biphasic increase in intracellular calcium for stimulation by pituitary adenylate cyclase activating peptide (PACAP). The initial spike is due to release of calcium from intracellular stores; the steady state is due to entry of calcium from the medium. Essentially all ECL cells respond to gastrin with a similar elevation of intracellular calcium $[Ca^{2+}]i$. The $EC_{50}$ for gastrin for both the calcium signal and histamine release is about $10^{-10}$ M. The elevation of steady state calcium is essential for release of histamine and is due to activation of receptor operated calcium channels (ROCC) by gastrin. The ECL cell also has voltage-dependent calcium channels (VDCC) (Zeng *et al.*, 1996). These VDCCs could be activated during exocytosis due to electrical changes following fusion

of the histamine containing vacuole with the plasma membrane of this cell. The histamine containing vacuole membrane has a V-type ATPase that is an electrogenic proton pump. Acidification by this pump depends on the presence of a chloride conductance that allows electrogenic proton pumping. Accumulation of histamine is driven by a histamine proton counter-transport mechanism as found in other amine transporting vacuoles (Loo, *et al.*, 1996). Whole cell patch clamp experiments have shown that the ECL cell has a resting voltage of about -50 mV and a low membrane conductance. Depolarization was found to activate $K^+$ channels that were $Ba^{2+}$ inhibited. Stimulation of histamine release by gastrin resulted in activation of a chloride current, presumably due to fusion of the vacuole membrane with the plasma membrane (Loo, *et al.*, 1996). The model of exocytosis that ensues from these data suggests that the chloride current derives from insertion of the $Cl^-$ channel present in the histamine containing vacuole. The depolarization that would result from this could be counteracted by the depolarization activated $K^+$ channels but could also activate VDCCs. Hence, the steady-state elevation of cell calcium by gastrin could be due to activation of both ROCC and VDCC pathways. The pathway for gastrin stimulation of parietal cell acid secretion is via CCK-B receptor activation on the ECL cell with release of histamine and activation of the $H_2$ receptor on the parietal cell. This is consistent with the ablation of acid secretion due to pentagastrin stimulation that is found with $H_2$ receptor antagonists (Lloyd *et al.*, 1992; Geibel *et al.*, 1995).

**b. Central Stimulation**

Carbachol causes a biphasic increase of intracellular calcium perhaps due to activation of an

$M_1$ or an $M_3$ receptor. MRNA for both has been found by RT/PCR, and blockade by pirenzepine indicates a functional $M_1$ receptor (Wilkes *et al.*,1991; Kajimura *et al.*, 1992). However, in the ECL cell population, only about 10 to 20 percent of the cells respond to carbachol (Zeng *et al.*, 1996). The parietal cell also has an $M_3$ receptor (Kajimura *et al.*, 1992), and from these data much of the vagal cholinergic response is due to direct activation of the parietal cell. Cimetidine was relatively ineffective in inhibition of carbachol-induced acid secretion, consistent with the distribution of muscarinic receptors in ECL and parietal cells. PACAP is a recent peptide found of the secretin/glucagon/VIP family (Andersson *et al.*, 1992). It is found in nerve fibres and a neurotransmitter. The PACAP receptor appears to be linked to both cAMP and calcium elevation in a variety of cells. Forskolin activates adenylate cyclase and is able to stimulate histamine release from ECL cells (Prinz *et al.*, 1993). PACAP is an effective and potent stimulant of elevation of intracellular calcium and of histamine release from the in vitro ECL cell preparation. The $EC_{50}$ for PACAP is about $10^{-9}$ M and is about 1,000-fold less than for VIP. The dose response to PACAP for the calcium signal is similar to the dose response for histamine release, which also illustrates that most of the ECL cells are PACAP responsive (Zeng *et al.*, 1996). The effect of PACAP on the fundic D cell is dominant when PACAP is present in the gastric circulation. PACAP has been shown to release somatostatin from D cells in vitro (Zeng *et al.*, 1996). Thus, the effect of PACAP on the ECL cell found in vitro in the absence of D cell stimulation corresponds to the effects of PACAP in vivo, provided that the effects of D cell stimulation are prevented. The effectiveness of PACAP as a stimulant of ECL cell function indicates that it is likely to be the central stimulant of the ECL cell. Epinephrine also stimulates histamine release from ECL

$M_1$ or an $M_3$ receptor. MRNA for both has been found by RT/PCR, and blockade by pirenzepine indicates a functional $M_1$ receptor (Wilkes *et al.*, 1991; Kajimura *et al.*, 1992). However, in the ECL cell population, only about 10 to 20 percent of the cells respond to carbachol (Zeng *et al.*, 1996). The parietal cell also has an $M_3$ receptor (Kajimura *et al.*, 1992), and from these data much of the vagal cholinergic response is due to direct activation of the parietal cell. Cimetidine was relatively ineffective in inhibition of carbachol-induced acid secretion, consistent with the distribution of muscarinic receptors in ECL and parietal cells. PACAP is a recent peptide found of the secretin/glucagon/VIP family (Andersson *et al.*, 1992). It is found in nerve fibres and a neurotransmitter. The PACAP receptor appears to be linked to both cAMP and calcium elevation in a variety of cells. Forskolin activates adenylate cyclase and is able to stimulate histamine release from ECL cells (Prinz *et al.*, 1993). PACAP is an effective and potent stimulant of elevation of intracellular calcium and of histamine release from the in vitro ECL cell preparation. The $EC_{50}$ for PACAP is about $10^{-9}$ M and is about 1,000-fold less than for VIP. The dose response to PACAP for the calcium signal is similar to the dose response for histamine release, which also illustrates that most of the ECL cells are PACAP responsive (Zeng *et al.*, 1996). The effect of PACAP on the fundic D cell is dominant when PACAP is present in the gastric circulation. PACAP has been shown to release somatostatin from D cells in vitro (Zeng *et al.*, 1996). Thus, the effect of PACAP on the ECL cell found in vitro in the absence of D cell stimulation corresponds to the effects of PACAP in vivo, provided that the effects of D cell stimulation are prevented. The effectiveness of PACAP as a stimulant of ECL cell function indicates that it is likely to be the central stimulant of the ECL cell. Epinephrine also stimulates histamine release from ECL

cells, as does the stimulant of adenylate cyclase, forskolin (Modlin and Tang, 1996). There is, therefore, evidence for four receptors in the ECL cell population isolated from rat gastric mucosa. The CCK-B and PACAP receptors are likely to be dominant in positive regulation of ECL function and, therefore, histamine dependent acid secretion. Much of cholinergic mediation of acid secretion is due to direct effects of acetylcholine on the parietal cell.

### 2.7.2 INHIBITION OF ECL CELLS

The setting of the rate of acid secretion to a specific level requires not only stimulation of the ECL cell but also inhibition. Various inhibitors of ECL cell calcium signalling and histamine releases have been described. A partial list includes somatostatin, PYY, galanin and even histamine. Two second messenger systems have been identified in ECL cells. The action of inhibitors of ECL cell function is exerted against calcium signalling by this cell. Inhibitors of ECL cell function apparently do inhibit calcium signalling.

#### a. Peripheral Inhibition of ECL Cells

Somatostatin inhibits ECL cell function by binding at an SST subtype 2 receptor. Type 3 and type 4 selective agonists, DC 25-12 and DC 32-92, and also somatostatin SS-14 required 100 to 1000 times' higher concentrations, namely $10^{-8}$ M. The effect of somatostatin was abolished by pre-incubation with pertussis toxin (PTX) showing that the SST 2 receptor in these cells was coupled to $G_i$ or $G_o$. The proximity of the fundic D cell to the ECL cell suggests that the somatostatin involved in ECL cell inhibition is released from the fundic, not the antral, D cell. Thus, regulation of the fundic D cell is intimately involved in the peripheral

regulation of ECL cell function. Somatostatin is so far the most effective inhibitory ligand of ECL cell calcium signalling and histamine release. It is the major candidate for the peripheral inhibitor of histamine release and, therefore, acid secretion. Agonists of fundic D cells, therefore, play an important role in the regulation of the ECL cell (Zeng and Sachs, 1998). The peptide PYY is found in duodenal extracts and has a variety of inhibitory actions. Gastrin stimulated histamine release was partially inhibited by PYY. There is an additive but not synergistic inhibitory effect of PYY and somatostatin on gastrin-stimulated histamine release (Prinz *et al.*, 1994; Zeng *et al.*, 1997). Evidence from the intact stomach and from isolated glands has shown that there is an $H_3$ histamine receptor subtype present with inhibitory pharmacological actions (Prinz *et al.*, 1993; Modlin and Tang, 1996). Earlier studies suggested that histamine secretion and especially histamine synthesis is under a feedback control of histamine. In the purified ECL cell preparation, the $H_3$ agonist, R-α-methylhistamine is able to inhibit gastrin-stimulated histamine release. The $H_3$-antagonist, thioperamide, is able to activate histamine release. These data suggest that there is a feedback loop to prevent excessive release of histamine by activation of the $H_3$ receptor on the ECL cell. There is additional evidence for an $H_1$ receptor on this cell type, but this would result in auto-activation of this rather toxic transmitter (Sachs and Prinz, 1996).

### b. Central (Neural) Inhibition of ECL Cells

Galanin is a 29-amino acid neuropeptide initially identified in the porcine intestine and now known to be widely distributed in peripheral and central neurons. In the periphery, galanin colocalizes with other neuropeptides (VIP, NPY) in nerve cell bodies and fibers of the

myenteric plexus and submucosal plexus close to the mucosal epithelium mucosa (Ekblad *et al.*, 1985). A recent study also showed that a galanin receptor (GALR1) was highly expressed in human gastric mucosal biopsies indicating that a target for galanin was present in the fundic mucosa (Lorimer and Benya, 1996). A second galanin receptor has also been cloned (GALR2) (Howard *et al.*, 1997). Among its many actions, galanin has been shown to influence gastric acid output (Yagci *et al.*, 1990). A direct action on antral G cell has been suggested because galanin was found to inhibit bombesin-stimulated gastric acid secretion in rats and dogs (Mungan *et al.*, 1992; Soldani *et al.*, 1988). Galanin inhibited basal and GRP-stimulated gastrin release in isolated stomach preparations as well as gastrin release from in vitro isolated rat G cells in primary culture (Schepp *et al.*, 1990). Since, it inhibits pentagastrin- stimulated and basal acid secretion (Smith *et al.*, 1997) and an abundance of galanin immunoreactive nerve endings are found in fundic mucosa (Ekblad *et al.*, 1985), galanin must act downstream of the G cell perhaps directly on fundic ECL cells. No effect of galanin on somatostatin release was found invivo and invitro, hence, somatostatin is not involved in the inhibitory action of galanin (Kwok *et al.*, 1988; Madaus *et al.*, 1988). Because galanin had no inhibitory effect on bethanechol or histamine-stimulated gastric acid secretion, a direct inhibitory action of galanin on parietal cell is also unlikely. Morphologically, gastric mucosal nerve terminals containing galanin are found adjacent to ECL cells in gastric fundic mucosa (Ekblad *et al.*, 1985). The peptide was found to inhibit gastrin stimulated calcium signals in the ECL cell. Here, 10 nM galanin reversibly blocked gastrin effects on ECL cell $[Ca^{2+}]_i$. The peptide also partially inhibits histamine release from the ECL cell with an $EC_{50}$ of $1 \times 10^{-10}$ M. The partial antagonist activity of galanin is due to a rapid desensitization of

the galanin receptor. Two galanin receptors have been cloned. They can be distinguished by the use of chimeric peptides containing the N-terminal sequence of galanin in combination with other peptide sequences. Galanin and galanin 1 receptor are present in the stomach. Galanin is, therefore, the only candidate identified thus far for central regulation of ECL cell function.

## 2.8 GASTRIC ACID SECRETION INHIBITORY MECHANISMS

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 ($H^+$) of the content of the antrum. Although, it has been suggested, that acid inhibition 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. 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). An auto regulatory mechanism of the antral content is low at different times of the day; this mechanism must 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 taken, 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 response 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). A variety of substances are known to inhibit gastric secretion when they come in contacts with the duodenal mucosa; examples include fat digestion products, acids and hypertonic solutions. Although, the effect of intravenous administration of cholecystokinin is to 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 manifest its full excitatory effects in the presence of cholecystokinin i.e. cholecystokinin denies receptor sites of gastrin (Thompson *et al.* 1987).

### 2.8.1 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 secretes 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 the 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.

## 2.9 DRUGS INHIBITING GASTRIC ACID SECRETION

Pharmacologically, several drugs have been shown 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:

a. Histamine $H_2$ receptor antagonists: cimetidine, ranitidine, famotidine, metiamide, burimamide, etc

b. Muscarinic $M_1$ receptor antagonists: pirenzipine

c. Gastrin $CCK_2$ receptors antagonists include: YF476, YM022, RPF3870, JB93182, AG041R, L-365,260 etc

d. Proton-pump inhibitors: omeprazole, lansoprazole, pantoprazole, etc

## 2.10 OPPOSING CENTRAL AND PERIPHERAL ACTIONS OF BRAIN-GUT PEPTIDES

One of the most intriguing findings that emerged from studies of central administration of peptides on gut function has been that some peptides have opposite central phenomenon. These have best been described as peptides that affect gastric secretion. However, it has also been noted that CCK and opiates have opposing central and peripheral effects on gastric emptying and pain modulation respectively, suggesting that it may be a more generalised occurrence than is currently recognised (Pappas *et al.*, 1985; Bechara *et al.*, 1985). This phenomenon shows the importance of the compartmentalisation provided by the blood-brain barrier. In general, the blood brain barrier limits diffusion of peptides from the peripheral circulation to the brain interstitial fluid and vice versa. However, in a number of areas, collectively known as the circumventricular organs, the blood-brain barrier is deficient permitting peripheral circulating peptides access to the central nervous system. Receptor binding studies have demonstrated receptors for many of the gut peptides in these areas, providing an anatomical substrate for feedback effects of circulating gut peptides on centrally controlled gut functions (Whitcomb *et al.*, 1990).

Binding of peptides that circulate peripherally to receptors in the circumventricular organs is probably an important pathway for feedback control of gut function through central pathways (McTigue *et al.*, 1995). Elucidation of the central pathway through which the brain regulates gastric function may lead to the development of new strategies for pharmacological manipulation of acid secretion and gastric motility.

## 2.11 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 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 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 (Dwork, 1982).

## 2.12 FUNCTIONS OF THE COMPONENTS OF GASTRIC JUICE

### a. Hydrochloric Acid

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

i. It establishes an extremely acidic environment in the stomach.

ii. It helps in the activation of pepsinogen to pepsin.

iii. It aids the destruction of microorganism ingested along with food.

iv. It provides an optimal low pH for peptic digestion.

v. Essential in gastric breakdown of connective tissue and muscle fiber (Ribbon *et al*, 1990).

**b. 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.

i. Pepsin is largely responsible for the stomach ability to initiate digestion of proteins.

ii. 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).

**c. Gastrin**

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

i. Control of acid secretion.

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

**d. 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).

## c. Mucus

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

i. Coats and lubricates the gastric surface

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

iii. Aids barrier function via gastric mucosal barrier.

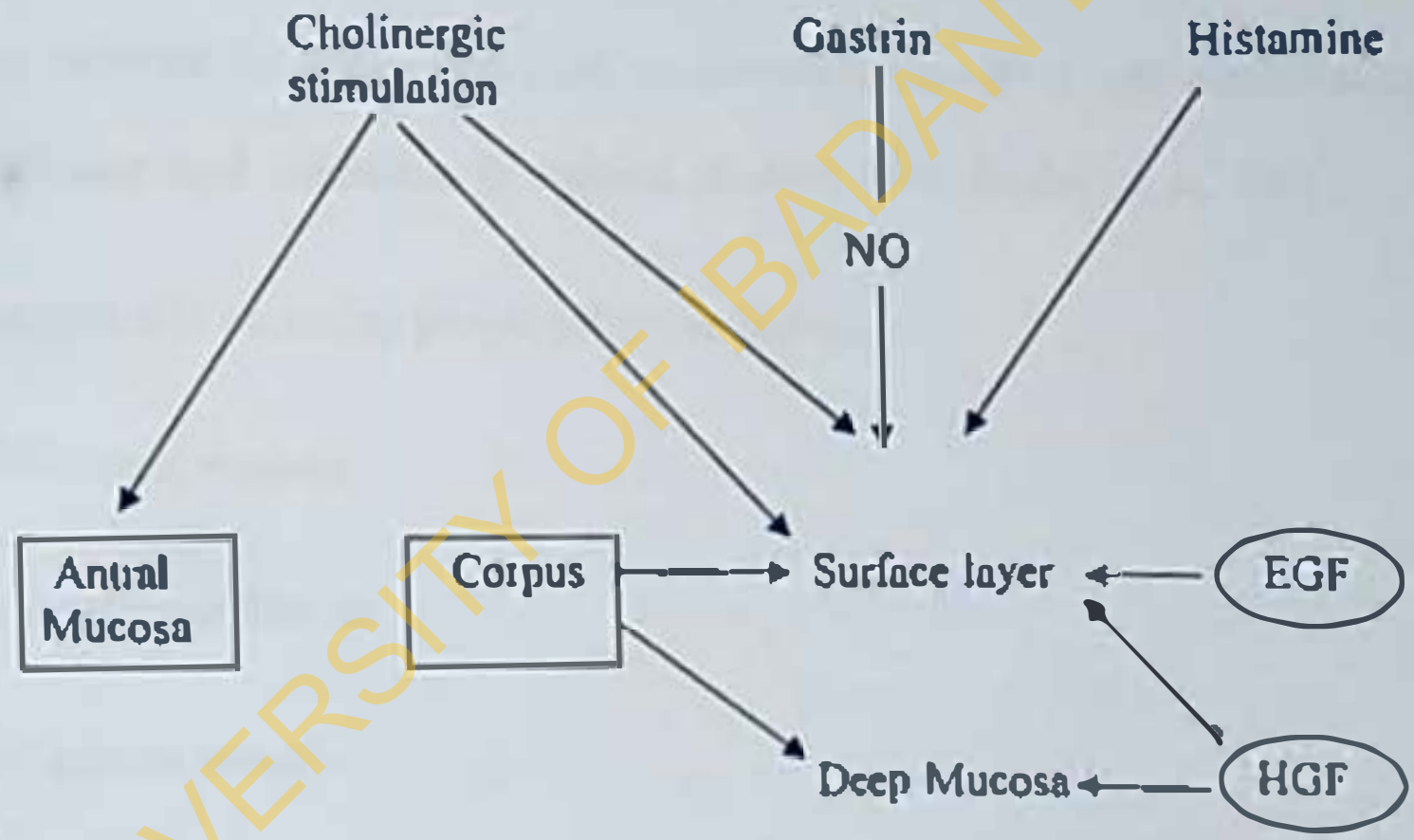
iv. Maintains a neutral pH of the plasma membrane epithelium. (Engle *et al*, 1995)

The luminal surface of the stomach is covered by a viscoelastic mucus gel acting as protective barrier when its composition is intact.

### i. Composition of gastric mucus

Mucus produced by mucus producing cells is a complex mixture containing mucin, water, electrolytes, sloughed off cells, enzymes and other materials like bacterial and its products (Hotta, 2000).

- Water content 90-95%
- Mucin 5-10% (aggregates of mucin forms mucus granules)
- Electrolytes ≥1%
- Others: Enzymes, Nucleic acid, Lipid, Plasma protein, secretory IgA, bacteria plus its products.




NO – Nitric oxide

ECF- Epidermal gro factor

Figure 5 Factors involved in the regulation of mucus synthesis (Ichikawa and Ishihara, 2011)

. Physiological importance of gastric mucus

. Maintains the lubrication of the gastric mucosa.

2. Covers the ingested food for the process of mixing and adhesion.

3. It helps for digestion.

4. It protects the surface epithelium from irritation by forming a thick mucus gel layer, that is, providing "gastroprotection".

Gastroprotection is a reduction or prevention of chemically induced acute haemorrhagic erosions by compounds such as prostaglandins (PG) and somatostatin (SH) derivatives without acid inhibition in rodents (Robert, 1979, Szabo *et al.* 1981).

This involves naturally occurring gastric defensive factors;

- Nature of gastric mucosa

- Gastric mucus metabolism

**iii. Nature of gastric mucosa**

This involves;

- The insoluble mucus gel layer adhering to the mucosal surface

- Solubilized form of the gastric lumen

**iv. Types of mucin cells.**

- Mucin from the surface (MUC5AC)

- Mucins from the gland mucus cells (MUC6)

They differ in peptide sequences and chemical composition of the carbohydrate moieties.

v. Regulatory factors (figure 5)

- increase in gastrin (Ichikawa *et al*, 1993)

- increase in histamine via peptide biosynthesis process of mucin)

- increase in carbachol via peptide biosynthesis and glycosylation step ( Ichikawa *et al.*, 1998 ).

- EGF and HGF.

- NO and Neuropeptides.

vi. Histamine ($H_2$)

They are drugs with heterocylic rings that is, possessed an aromatic ring with a flexible chain joined to a polar group (Table 2). They are basically used for the treatment of gastritis and gastric ulcer. Those compounds are either five membered first generation or six membered second generation aromatic ring series ( Fukushima *et al*. 2006; Harada *et al*. 2007; Murashima *et al*, 2009; Ichikawa *et al*, 2009a ).

1st generation $H_2$ Blockers: Cimetidine and Ranitidine

2nd generation $H_2$ Blockers: Lafutidine and Roxatidine.

**Table 2** $H_2$ Blocking drugs that enhance mucus activity

| Activity | $H_2$ Blockers | |
|---|---|---|
| | Ist generation | 2nd generation |
| (-) Gastric acid | √√ | √√ |
| (+) Gastric mucus | x | √√ |
| Neutralizing agent induced gastric damage protection | x | √√ |

x= block √√ = enhance

## 2.13 EFFECTS OF UNDER SECRETION OF GASTRIC JUICE

### 2.13.1 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).

### 2.13.2 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 activities. However, hypochlorhydria is a condition of diminished acid secretion (Guyton *et. al.*, 2000). Moreover, the overall digestion of food in the entire gastrointestinal tract is still almost normal even with achlorhydria. Thus, the symptomology of achlorhydria is limited to the stomach (Guyton *et al*, 2000).

### 2.13.3 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 anaemia. 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 anaemia (Toh *et al*, 1997). In addition, pernicious anaemia 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 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).

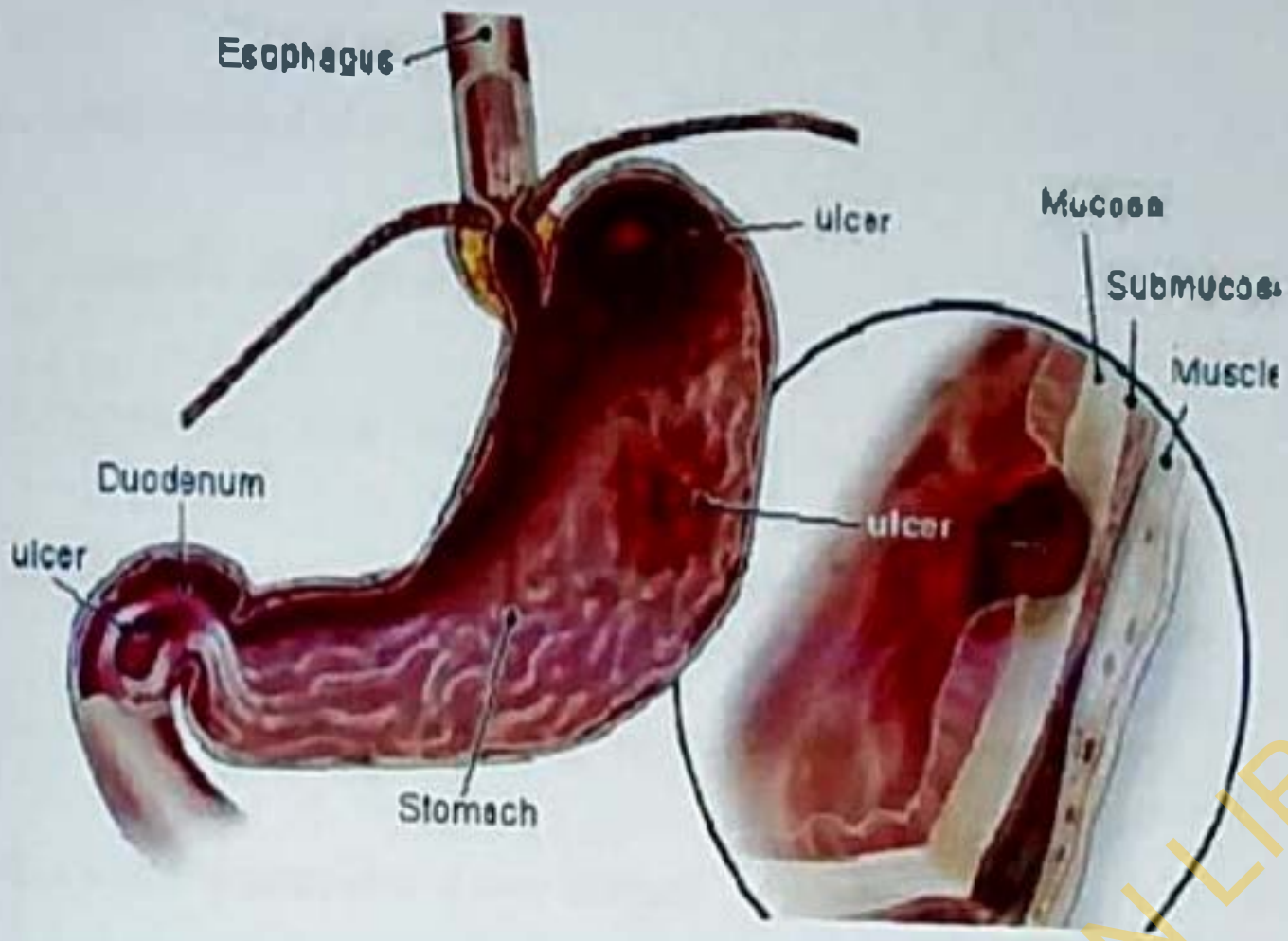
## 2.14 EFFECTS OF EXCESSIVE GASTRIC SECRETION

### 2.14.1 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 inflammatioo 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 bacterial 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 ($H^+$) then diffuse into the stomach epithelium creating an additional havoc that leads 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).

### 2.14.2 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). 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 (Figure 6). Peptic ulcer will develop when the summation of these forces was resolved in favour of the aggressive factor, being the gastric hydrochloric acid, HCl (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 (Acird *et al*, 1953; Mc Quaid *et al*, 1992).



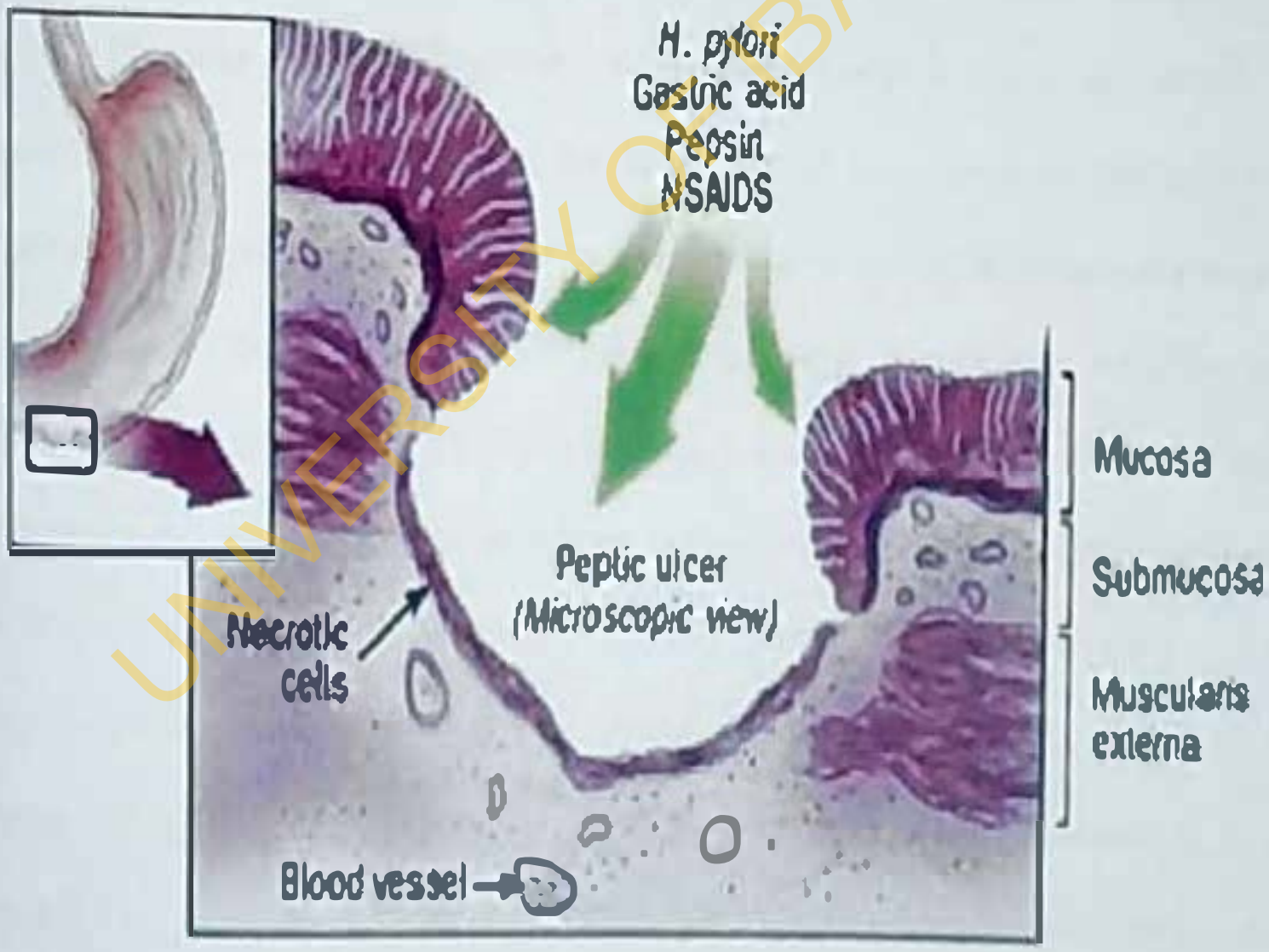




Figure 6 Diagram of gastric ulcer formation (GI Consult, 2007)

a. Classification of Peptic ulcer

i. Stomach (called gastric ulcer)

ii. Duodenum (called duodenal ulcer)

iii. Oesophagus (called Oesophageal ulcer)

iv. Meckel's Diverticulum (called Meckel's Diverticulum ulcer)

b. The Pathogenesis of Ulcer Disease

Early in this century, the inevitable association between the presence of acid and disease was recognized (Schwartz, 1910). However, since almost everyone secretes acid and few get sick, clearly this was not the full answer. Resistance to acid is a property of this region of the gastrointestinal tract. Apparently, the apical facing membranes of the cells, other than the parietal cell, have no proteins able to transport protons, and therefore, are essentially proton impermeable. Since the hydrophilic mucous gel layer cannot form a barrier to proton back diffusion and the rate of $HCO_3^-$ secretion cannot exceed more than 10 percent of acid secretion, the defense against acid back diffusion must lie in the tight junctions between the epithelial cells. In 1983, it was suggested that a bacterial infection by an organism, Helicobacter pylori (H. pylori), was essential for the development of duodenal and gastric ulcer (Marshall, 1983). Subsequent studies have shown that this seminal observation was correct. H. pylori do not play a role in esophagitis but does also result in gastritis (Flytgat et al., 1993). Eradication of H. pylori has been shown to greatly reduce the incidence of ulcer recurrence (Malfertheiner and Ditschuneit, 1990). Both acid and H. pylori are essential for

peptic ulcer disease. It is tempting to speculate that the contribution of H. pylori is mediated via tight junction disruption. As the tight junctions become progressively leakier, in the presence of luminal acid, acid back diffusion increases. In contrast to the apical surface, the basal lateral membranes of the gastric and duodenal epithelium have the normal high proton permeability of mammalian cell membranes. The cell cytoplasm will then acidify, the cell die and increasing acid back diffusion result. Besides acid back diffusion, other molecules probably can back diffuse, such as the bacterial urease. This, in turn, will set up an immune response such as gastritis. Since normally the esophageal epithelium is not exposed to acid, neither the cells nor the tight junctions are constructed to resist acid. Thus, H. pylori do not contribute to acid related disease in this area of the body. A yet unresolved problem is why so many people with both acid secretion and H. pylori infection do not get gastric and duodenal ulcers. Perhaps, different strains of H. pylori differ in their ability to induce tight junction damage. Perhaps, other factors predispose to H.pylori induced tight junction damage (Sachs *et al.*, 1994).

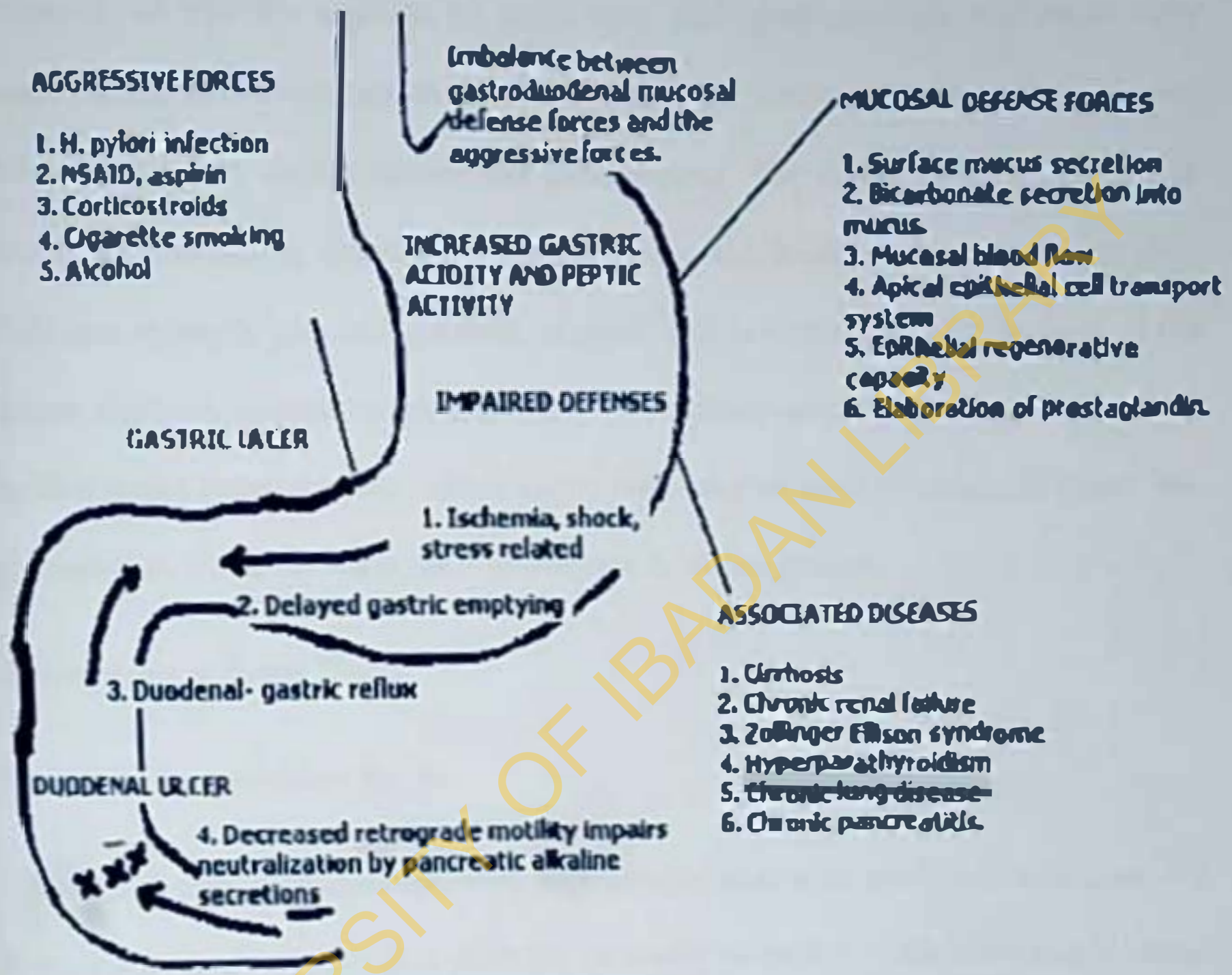



Figure 7 Various factors implicated in peptic ulcer (Cao *et al.*, 2005)

### c. 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 a 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.

### d. 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); 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 oesophagus from severe/continuing vomiting.

- Melena (tarry, foul-smelling faeces due to oxidized iron from haemoglobin);

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

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

### f. Gastric ulcer models and therapy

Gastric erosions and ulcers are induced by various factors (figure 7) including gastric oversecretion and retention (Rao *et al*, 2004; Cao *et al*, 2005), weakening and depleting agents of mucin layer, mucosal injury and inflammation (Wallace and Granger, 1996; Neal, 2003; Isobe *et al*, 2004; Byun *et al*, 2007). Other ulcer inducing agents also include;

- non-steroidal anti-inflammatory drugs (NSAIDs) that block production of prostaglandins (Slomiany *et al*, 1997; Filaretova *et al*, 2002; Cao *et al*, 2004; Rao *et al*, 2004; Kim *et al*, 2005)
- stresses (Cao *et al*, 2004; Rao *et al*, 2004; Byun *et al*, 2007)
- alcohols (Cao *et al*, 2004; Rao *et al*, 2004; Raffin *et al*, 2007)
- cigarettes, trauma, sepsis and shock (Mózsik and Jávor, 1988; Davies *et al*., 1994; Ding *et al*., 1998; Hooderwerf and Pasricha, 2006)
- gastric hypermotility and acetic acid accumulation (Dias *et al*. 2000; Rao *et al*, 2004; Cantarella *et al*, 2005; Isbil *et al*, 2006; Cantarella *et al*, 2007) and
- Helicobacter pylori infection (Wallace and Granger, 1996; Neal, 2003).

However, in the therapy of gastric ulcers, proton pump inhibitors that block acid secretion from parietal cells, antacids, histamine receptor ($H_2$) antagonists, prostaglandins that strengthen mucin layer, and antibiotics to eliminate *H.pylori* have been used (Wallace and Granger, 1996; Neal, 2003).

## 2.15 REACTIVE OXYGEN SPECIES (ROS)

Reactive oxygen species are

- the superoxide anion which is both ion and radical.
- radicals like the hydroxyl radical. It is the most reactive of them all; note how it differs from the hydroxyl ion
- molecules like hydrogen peroxide

- ions like the hypochlorite ion

A radical (also called a "free radical") is a clusters of atoms one of which contains an unpaired electron in its outermost shell of electrons. This is an extremely unstable configuration and radicals quickly react with other molecules or radicals to achieve the stable configuration of 4 pairs of electrons in their outermost shell (one pair for hydrogen) (Guzik *et al*., 2003).

## 2.15.1 ROS FORMATION

Reactive oxygen species are formed by several different mechanisms:

- the interaction of ionizing radiation with biological molecules
- as an unavoidable byproduct of cellular respiration. Some electrons passing "down" the electron transport chain leak away from the main path (especially as they pass through ubiquinone) and go directly to reduce oxygen molecules to the superoxide anion .
- synthesized by dedicated enzymes in phagocytic cells like neutrophils and macrophages
  - NADPH oxidase (in both type of phagocytes)
  - myeloperoxidase (in neutrophils only)

## 2.15.2 ROS ACTIVITY

Strong oxidants like the various ROS can damage other molecules and the cell structures of which they are a part. Among the most important of these are the actions of free radicals on

the fatty acid side chains of lipids in the various membranes of the cell, especially mitochondrial membranes (which are directly exposed to the superoxide anions produced during cellular respiration).

A hydroxyl radical removes a hydrogen atom from one of the carbon atoms in the fatty acid chain (only a portion of which is shown) forming

- a molecule of water and leaving the carbon atom with an unpaired electron thus now a radical.
- Several possible fates await it.

One of the most likely (and shown here) is to react with a molecule of oxygen ($O_2$) forming a peroxyl radical.

This might then steal a hydrogen atom from a nearby side chain making it now a radical.

One of the insidious things about free radicals is that in interacting with other molecules to gain a stable configuration of electrons, they convert that target molecule into a radical. So a chain reaction begins that will propagate until two radicals meet each other and each contributes its unpaired electron to form a covalent bond linking the two.

## 2.15.3 TWO COMMON EXAMPLES

The peroxyl radical may interact with:

- another peroxyl radical on a nearby side chain crosslinking them with a covalent bond.
- another nearby carbon-centered radical crosslinking them covalently.

In both these latter cases, radical formation comes to an end but with the result that the fatty acid side chains of membrane lipids may have become so deformed as to damage the membrane. The lipofuscin so characteristic of aging cells may be formed by these mechanisms

## 2.15.4 DEFENSES AGAINST ROS

Cells have a variety of defenses against the harmful effects of ROS. These include two enzymes:

- **superoxide dismutase (SOD),** which converts two superoxide anions into a molecule of hydrogen peroxide and one of oxygen, and
- **catalase**

as well as several small molecules that are antioxidants, such as

- **alpha-tocopherol (vitamin E).** This can break the covalent links that ROS have formed between fatty acid side chains in membrane lipids.

- uric acid. (Perhaps the long life span of some reptiles and birds is attributable to their high levels of uric acid.)
- vitamin C (in the right concentration) (Pignatelli *et al.*, 1998; Guzik *et al.*, 2003,).

### 2.15.5 ROS ARE ESSENTIAL

But it is important that the attempt to limit the production of ROS have not succeed too well, because ROS have important functions to perform in the cell.

Examples:

- The cells of the thyroid gland must make hydrogen peroxide in order to attach iodine atoms to thyroglobulin in the synthesis of thyroxine.
- Macrophages and neutrophils must generate ROS in order to kill some types of bacteria that they engulf by phagocytosis.
  - Bacteria are engulfed into a phagosome.
  - This fuses with a lysosome.
  - Subunits of the enzyme NADPH oxidase assemble in the lysosome membrane forming the active enzyme.
  - It catalyzes the synthesis of the superoxide anion.

    $$NADPH - 2\,e^- + 2O_2 \longrightarrow NADP^+ + H^+ + 2\cdot O_2^-$$

  - This activity produces a large increase in oxygen consumption, called the "respiratory burst" (Hyun *et al.*, 2013).
  - Superoxide dismutase (SOD) converts this into hydrogen peroxide, which kills

- off the engulfed bacteria (except those that manufacture enough catalase to protect themselves). (Krotz *et al.*, 2002).

Neutrophils (but not macrophages) also kill off engulfed pathogens by using the enzyme myeloperoxidase which catalyzes the reaction of hydrogen peroxide (made from superoxide anions) with chloride ions to produce the strongly antiseptic hypochlorite ion. ($OCl^-$).

$$H_2O_2 + Cl^- \longrightarrow HOCl \text{ (hypochlorous acid)} + OH^-$$

$$HOCl \longrightarrow H^+ + OCl^- \text{ (Sen, 2003).}$$

## 2.16 INDOMETHACIN

### 2.16.1 History

Indomethacin was discovered in 1963 (Hart and Boardman, 1963) and it was first approved for use in the U.S. by the Food and Drug Administration in 1965. Its mechanism of action, along with several other NSAIDs that inhibit COX has been described (Ferreira *et al.*, 1971). Indomethacin, also known chemically as 2-{1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl}acetic acid with the chemical formula, $C_{19}H_{16}ClNO_4$ has a molecular mass of 357.787 $g.mol^{-1}$.

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling. It works by inhibiting the production of prostaglandins, molecules known to cause these symptoms (TOA, 1999).

**a. Clinical indications**

The clinical indications include; Hemicrania continua, ankylosing spondylitis, arthritic gout, Bartter syndrome, bursitis, cryoglobulinemia, dysmenorrhea (menstrual cramps), exertion headache, fever and pain associated with malignant diseases, hemicrania continua, hypnic headache, juvenile arthritis, migraine, patent ductus arteriosus, nephrogenic diabetes insipidus, osteoarthritis, Paget's disease of bone, paroxysmal hemicrania, pericarditis, primary stabbing headaches, pseudogout, psoriatic arthritis, Reactive arthritis, renal colic (pain due to kidney stones), retinopathy of prematurity, rheumatoid arthritis, tendinitis, trigeminal autonomic cephalgias, Headaches resulting from Valsalva maneuver (Smyth *et al.*, 2004; Garza and Schwedt 2013; Hemicrania Continua, 2014; Medication for Migraine, 2014).

Indomethacin has also been used clinically to delay premature labor, reduce amniotic fluid in polyhydramnios, and to close patent ductus arteriosus. Indomethacin is a potent drug with many serious side effects and not good enough as analgesic for minor aches and pains or fever. It is better described as an anti-inflammatory, rather than an analgesic.

**b. Contraindications**

Indomethacin is contraindicated for the following: recurrent peptic ulcer, or history of ulcer disease, allergy to indomethacin, aspirin, or other NSAIDs, patients with nasal polyps reacting with an angioedema to other NSAIDs, children under 2 years of age (with the exception of neonates with patent ductus arteriosus), severe pre-existing renal and liver damage, caution: pre-existing bone marrow damage (frequent blood cell counts are

indicated), caution: bleeding tendencies of unknown origin (indomethacin inhibits platelet aggregation), caution: Porkinson's disease, epilepsy, psychotic disorders (indomethacin may worsen these conditions), concurrent with potassium sparing diuretics, patients who have a patent ductus arteriosus dependent heart defect (such as Transposition of the great vessels) and significant hypertension (Giles and Bisits, 2007; Indomethacin, 2013; Garza and Schwedt, 2013; Medication for Migraine, 2014)

c. Mechanism of action

Indomethacin is a non-selective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes that participate in prostaglandin synthesis from arachidonic acid. Prostaglandins are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation. Prostaglandins also cause uterine contractions in pregnant women. Indomethacin is an effective tocolytic agent able to delay premature labour by reducing uterine contractions through inhibition of PG synthesis in the uterus and possibly through calcium channel blockade.

Indomethacin has two additional modes of actions with clinical importance:

i. It inhibits motility of polymorphonuclear leukocytes

ii. It uncouples oxidative phosphorylation in cartilaginous (and hepatic) mitochondria, like salicylates. These additional effects help in accounting for the analgesic and the anti-inflammatory properties. Indomethacin readily crosses the placenta and can reduce foetal urine production to treat polyhydramnios. It does so by reducing renal blood flow and increasing renal vascular resistance, possibly by enhancing the effects of vasopressin on the foetal kidneys (Ferreira *et al*., 1971; Giles and Bisits, 2007)

**d. Adverse effects**

Since, indomethacin inhibits both COX-1 and COX-2, it prevents the production of prostaglandins in the stomach and intestines, which maintain the mucous lining of the gastrointestinal tract. Indomethacin, therefore, like other non-selective COX inhibitors can cause peptic ulcers. These ulcers can result in serious bleeding and/or perforation requiring hospitalization of the patient. Indomethacin also reduces plasma rennin activity and aldosterone levels, and increases sodium and potassium retention. It also enhances the effects of vasopressin. Due to its strong antipyretic activity, indomethacin may obscure the clinical course of serious infections. Psychosis has also been reported with its prolonged use. The frequency and severity of side effects and the availability of better tolerated alternatives make indomethacin today a drug of second choice. Its use in acute gout attacks and in dysmenorrhoea is well-established because in these indications, the duration of treatment is limited to a few days only, therefore serious side effects are not likely to occur.

**e. Animal toxicity and human overdose**

Indomethacin has a high toxicity profile both for animals (in rats, 12 mg/kg) and for humans. Generally, overdose in humans causes drowsiness, dizziness, severe headache, mental confusion, paresthesia, numbness of limbs, nausea and vomiting. Severe gastrointestinal bleeding is also possible. Cerebral oedema and cardiac arrest with fatal outcome have been seen in children. The treatment is symptomatic and largely the same as with diclofenac. However, the possibility of severe GI tract symptoms should be particularly noted. The risk of overdose after exaggerated local treatment with gel or spray is very limited (Brayfield, 2014).

## 2.17 EXPERIMENTAL INDOMETHACIN-INDUCED PEPTIC 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 40 mg/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 carbohydrate component of the gastric mucosal (Menguy and Desbaillet, 1969). 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.

## 2.18 MECHANISM OF NSAID's ULCER FORMATION

Gastroduodenal ulceration and bleeding are the major limitations to the use of non-steroidal anti-inflammatory drugs (NSAIDs). The development of safer NSAIDs or of effective therapies for the prevention of the adverse effects of existing NSAIDs requires a better understanding of the pathogenesis of NSAID-induced ulcer disease. NSAIDs can cause damage to the gastroduodenal mucosa via several mechanisms, including the topical irritant

effect of these drugs on the epithelium, impairment of the barrier properties of the mucosa, suppression of gastric prostaglandin synthesis, reduction of gastric mucosal blood flow and interference with the repair of superficial injury. The presence of acid in the lumen of the stomach also contributes to the pathogenesis of NSAID-induced ulcers and bleeding. by impairing the restitution process, interfering with haemostasis and inactivating several growth factors that are important in mucosal defence and repair. The ability of non-steroidal anti-inflammatory drugs (NSAIDs) to cause ulceration and bleeding in the upper gastrointestinal tract was first documented by the endoscopic study of Douthwaite and Lintott in 1938. They demonstrated the ability of aspirin to damage the stomach. In general, the properties of NSAIDs that contribute to ulcerogenesis can be divided into two categories: (1) topical irritancy, and (2) the suppression of prostaglandin synthase activity. In addition, the presence in the stomach and duodenum of acid and, in some cases, Helicobacter pylori (H. pylori), may contribute to the ability of NSAIDs to damage the mucosa.

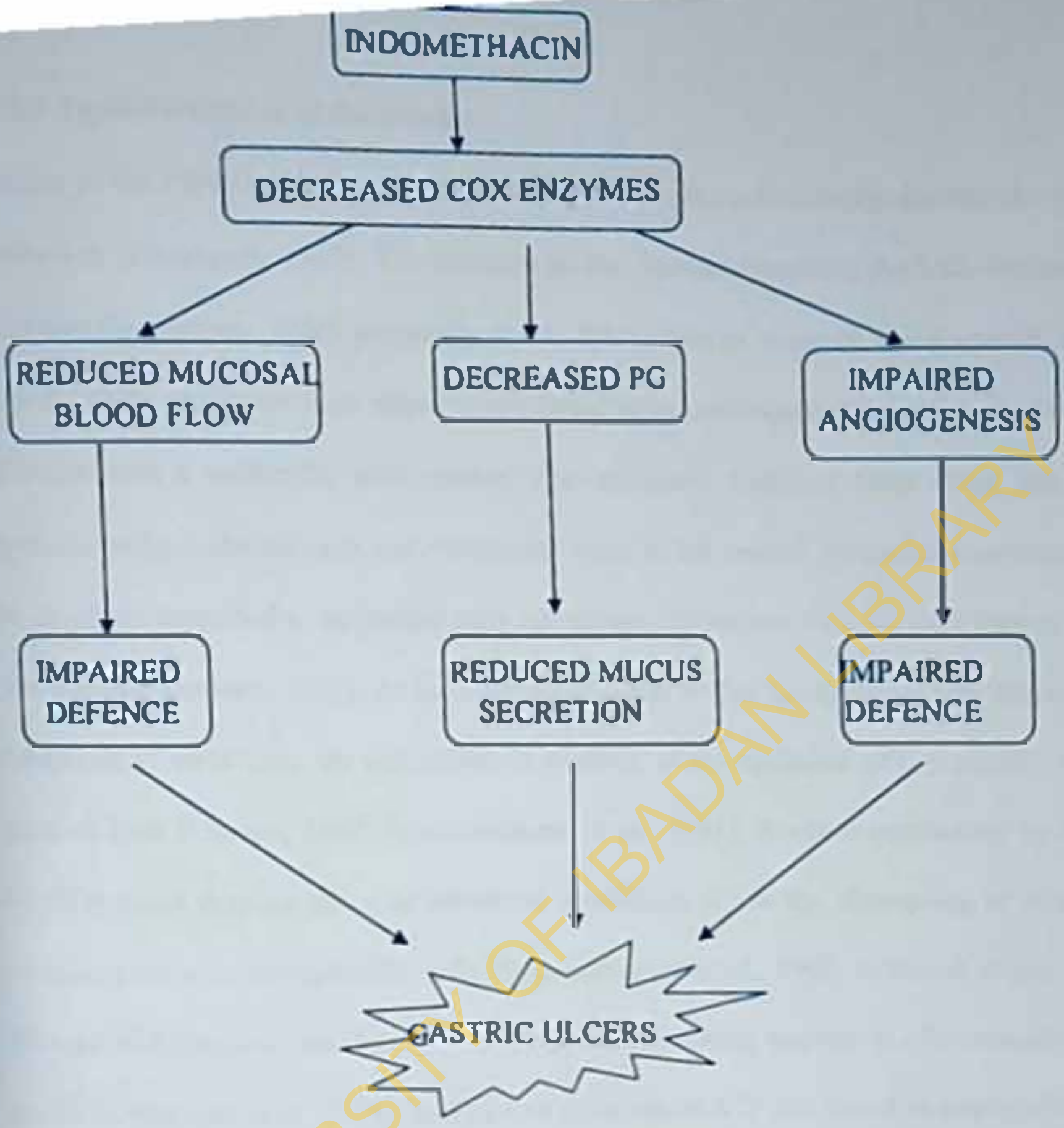



Figure 12 Indomethacin-induced ulcers (Amita *et al*., 2012).

COX - cyclooxygenase

PG - prostaglandins

### 2.18.1 Topical irritation of the mucosa

Studies in the 1960s by Davenport suggested that aspirin could directly damage the gastric epithelium (Davenport, 1969). The breaking of the 'barrier' permitted the back-diffusion of acid into the mucosa, which eventually led to the rupture of mucosal blood vessels. These topical irritant properties were subsequently found to be predominantly associated with those NSAIDs with a carboxylic acid residue. The unionized forms of these drugs can enter epithelial cells in the stomach and duodenum. Once in the neutral intracellular environment, the drugs are converted to an ionized state and cannot diffuse out. This has been referred to as 'ion trapping' (Fromm, 1987). As the drug accumulates within the epithelial cell, the osmotic movement of water into the cell results in swelling of the epithelial cell, eventually to the point of lysis (Fromm, 1987; Somasundaram *et al.*, 1995). Another mechanism by which NSAIDs could damage the gastroduodenal epithelium is via the uncoupling of oxidative phosphorylation in the epithelial cells (Somasundaram *et al.*, 1995; Mahmud *et al.*, 1996). Various NSAIDs have been shown to uncouple mitochondrial respiration ( Somasundaram *et al.*, 1995; Mahmud *et al.*, 1996), leading to a depletion of ATP and therefore a reduced ability to regulate normal cellular functions, such as the maintenance of intracellular pH.

The ability of NSAIDs to uncouple oxidative phosphorylation also appears to be related to some extent to acidic moieties (such as carboxylic acid residues), since substitution at these sites interferes with the ability of these compounds to act as uncouplers (Mahmud *et al.*, 1996). Erosions and ulcers can also be produced in experimental circumstances in which NSAIDs are administered parenterally (Wallace and McKnight, 1993). On the other hand, the small intestine would be repeatedly exposed to NSAIDs that are excreted in bile and recycled

enterohepatically, so it is conceivable that the uncoupling of oxidative phosphorylation is an important component of the pathogenesis of NSAID-induced enteropathy.

A third mechanism that could account for the topical irritant properties of NSAIDs is their ability to decrease the hydrophobicity of the mucus gel layer in the stomach. Lichtenberger and co-workers have proposed that this layer is a primary barrier to acid-induced damage in the stomach (Goddard *et al.*, 1987; Lichtenberger, 1995), having demonstrated that the surface of the stomach is hydrophobic and that this hydrophobicity can be reduced by various pharmacological agents. For example, NSAIDs were shown to associate with the surface-active phospholipids within the mucus gel layer, thereby reducing its hydrophobic properties (Goddard *et al.*, 1987; Lichtenberger *et al.*, 1995a, b). These investigators further demonstrated that the mucus gel layer in the stomach of rats and mice given NSAIDs was converted from a non-wettable to a wettable state. This effect was found to persist for several weeks or months after the cessation of NSAID administration (Lichtenberger, 1995; Lichtenberger *et al.*, 1995a).

### 2.18.2 Suppression of prostaglandin synthesis

Vane's discovered in 1971 that NSAIDs inhibit prostaglandin synthesis (Vane, 1971). This led to the discovery of the ability of minute quantities of exogenous prostaglandins to protect the gastrointestinal tract from injury induced by topical irritants and NSAIDs (Robert *et al.*, 1976). This in turn led to extensive research into the roles of prostaglandins in mucosal defence. There is substantial evidence that the ability of an NSAID to cause gastric damage correlates well with its ability to suppress gastric prostaglandin synthesis (Whittle, 1981,

Rainsford and Willis, 1982; Lanza, 1989; Wallace *et al.*, 1993), agents that are weak inhibitors of gastric prostaglandin synthesis are less ulcerogenic (Whittle, 1981, Wallace *et al.*, 1993). There is also a good correlation between the time- and dose-dependency of suppression of gastric prostaglandin synthesis by NSAIDs and their ability to cause gastric ulcers (Whittle, 1981; Rainsford and Willis, 1982; Lanza, 1989). Endogenous prostaglandins are involved in the regulation of mucus and bicarbonate secretion by the gastric and duodenal epithelium, mucosal blood flow, epithelial cell proliferation, epithelial restitution and mucosal immunocyte function (Wallace and Tigley, 1995).

Prostaglandins are not the only endogenous substances regulating these components of mucosal defence; indeed, nitric oxide appears to perform many of the same functions as the prostaglandins in this respect (Wallace and Tigley, 1995). Thus, the inhibition of prostaglandin synthesis alone may not result in the formation of gastric erosions or ulcers. For example, mice in which the gene for cyclo-oxygenase-1 was disrupted exhibited negligible levels of gastric prostaglandin synthesis yet did not spontaneously develop gastric erosions or ulcers (Langenbach *et al.*, 1995).

The suppression of mucosal prostaglandin synthesis, while not necessarily resulting in ulcer formation, will reduce the ability of the gastric mucosa to defend itself against luminal irritants. This has been demonstrated very clearly in experimental animals. Doses of NSAIDs that substantially reduced mucosal prostaglandin synthesis did not cause overt gastric injury but greatly increase the susceptibility of the gastric mucosa to damage induced by irritants e.g. bile salts (Whittle 1977). The fact that gastric ulcers can be induced by parenterally administered NSAIDs (Estes *et al.*, 1993; Henry *et al.*, 1993; Wallace and McKnight, 1993),

and that this damage can be produced independently of the presence of luminal acid (Rashid and Toffolon, 1993; Janssen *et al.*, 1994) suggests that the impairment of mucus and/or bicarbonate secretion may be of lesser significance. While prostaglandins are potent inhibitors of mast cell degranulation (Hogaboam *et al.*, 1993), mast cells are capable of releasing a variety of mediators (e.g. leukotriene C4 and platelet- activating factor) that can contribute to mucosal injury (Rosam *et al.*, 1986; Rioux and Wallace, 1994), the effects of NSAIDs on mast cells do not seem to be crucial to the pathogenesis of mucosal injury. This latter conclusion is based on the observation that rats in which mucosal mast cells have been depleted by chemical means, or mice that are genetically deficient of mast cells, exhibit similar susceptibility to NSAID-induced gastric injury as do their respective controls (Rioux and Wallace, 1996).

The rate of epithelial turnover has been shown to be reduced by NSAIDs and could contribute to the pathogenesis of ulcer disease (Eastwood and Quimby, 1982). However, it is unlikely that this effect is the principal one that leads to the development of an ulcer.

The component of mucosal defence that appears to be most profoundly altered by NSAIDs is the gastric microcirculatory response to injury. When the mucosa is exposed to an irritant, or when superficial epithelial injury occurs, mucosal blood flow substantially increases. This is probably a response aimed at removing any toxins or bacterial products that enter the lamina propria, neutralizing back-diffusing acid and contributing to the formation of a microenvironment at the surface of the mucosa that is conducive for repair (Wallace and Granger, 1996). As described below, NSAIDs can reduce gastric mucosal blood flow and profoundly alter the behaviour of neutrophils flowing through the gastric microcirculation.

### 2.18.3 Effects on the microcirculation

The ability of NSAIDs to reduce gastric mucosal blood flow has been recognized for several decades (Ashley *et al.*, 1985; Kitahora and Guth, 1987; Gana *et al.*, 1987). Prostaglandins of the E and I series are potent vasodilators that are continuously produced by the vascular endothelium, so the inhibition of their synthesis by an NSAID leads to a reduction in vascular tone. Several lines of evidence have suggested that damage to the vascular endothelium is an early event following the administration of NSAIDs to experimental animals (Rainsford, 1983; Wallace *et al.*, 1990). Endothelial injury is also an early event in the pathogenesis of gastrointestinal damage associated with ischaemia- reperfusion (Granger *et al.*, 1930), in which neutrophils have been demonstrated to play a critical role as mediators of endothelial injury (Hernandez *et al.*, 1987). This observation led to the possible role of the neutrophils in the pathogenesis of experimental NSAIDs gastropathy.

Studies had demonstrated that NSAIDs administration to rats resulted in a rapid and significant increase in the number of neutrophils adhering to the vascular endothelium in both gastric and mesenteric venules (Asako *et al.*, 1992a, 1992b; Wallace *et al.*, 1993a; Wallace *et al.* 1993b). This effect was typically seen within 15 - 60 minutes after the administration of an NSAIDs, consistent with the period of time required for the suppression of prostaglandin synthesis by these drugs (Wallace and McKnight, 1990). Subsequent studies demonstrated that this adherence was dependent on the expression of the B2-integrins (CD11/CD18) on the neutrophils and intercellular adhesion molecule-I (ICAM-1) on the vascular endothelium (Wallace *et al.* 1993b). Such adherence could also be demonstrated to occur in vitro using isolated human neutrophils and endothelial cells from human umbilical vein (Yoshida *et al.*,

1993). Furthermore, the upregulation of ICAM-1 on the vascular endothelium in the gastric microcirculation was shown to occur within 15 - 30 minutes of the administration of an NSAIDs to rats, and this could be prevented by the administration of exogenous prostaglandins (Andrews *et al.*, 1994).

Administration of prostaglandins at doses previously shown to prevent gastric injury inhibited NSAID-induced leukocyte adherence (Asako *et al.* 1992a, Asako *et al.* 1992b). Further evidence for a link between neutrophil adherence and NSAID-induced gastric injury came from studies of arthritic rats (McCafferty *et al.*, 1995). These animals, like humans with rheumatoid arthritis, exhibited an increased susceptibility to NSAID-induced gastric damage. Interestingly, these rats also exhibited an increased level of neutrophils adherence to the vascular endothelium. This appeared to be due to an increase in expression of ICAM-1 on the vascular endothelium of arthritic rats, since pre-treatment with an antibody against ICAM-1 reduced leukocyte adherence and the susceptibility to NSAID-induced gastric damage to levels seen in healthy controls (McCafferty *et al.*, 1995). These studies suggest that in response to the suppression of prostaglandin synthesis by NSAIDs, there is a rapid upregulation of expression of ICAM-1 on the vascular endothelium and possibly an upregulation of b2 integrin expression on circulating neutrophils, resulting in an increased adherence of neutrophils to the endothelium. Also it has been suggested that tumour necrosis factor-a (TNFα) might be the key signal for NSAID-induced neutrophils adherence within the gastric microcirculation (Santucci *et al.*, 1994). The release of TNFα from macrophages and mast cells has been shown to be suppressed by prostaglandins (Kunkel *et al.*, 1986; Hogaboam *et al.* 1993), and TNFα is a well-characterized stimulus for adhesion molecule

expression (Rothlein *et al.*, 1988). It has been demonstrated that the levels of TNFα in the plasma of rats significantly increased following the administration of indomethacin, this being accompanied by a parallel accumulation of neutrophils within the gastric microcirculation and the development of gastric injury. Furthermore, pre-treatment with a TNFα synthesis inhibitor, pentoxifylline, dose-dependently reduced neutrophils accumulation in the gastric microcirculation and gastric damage (Santucci *et al.*, 1994). These results have been confirmed and extended by using a number of structurally unrelated inhibitors of TNFα synthesis and an anti-TNFα antibody (Appleyard *et al.*, 1996).

Another group of mediators that might contribute to the increase in neutrophils adherence following NSAID administration is the leukotrienes. Like prostaglandins, leukotrienes are derived from arachidonic acid and have been shown to be capable of altering the susceptibility of the gastric mucosa to injury (Rainsford, 1987; Piper *et al.*, 1988; Asako *et al.*, 1992a; Vaananen *et al.*, 1992), at least in part through stimulatory effects on neutrophil adherence to the vascular endothelium (Asako *et al.* 1992a). Inhibitors of leukotriene synthesis and leukotriene receptor antagonists have been shown to exert some protective effects in experimental models of NSAID-induced gastric damage (Rainsford, 1987; Piper *et al.*, 1988; Vaananen *et al.*, 1992). There is also evidence of an elevated leukotriene B4 production following NSAID administration to laboratory animals (Asako *et al.* 1992a) and man (Hudson *et al.*, 1993), and inhibitors of leukotriene synthesis and a leukotriene B4 receptor antagonist have been shown to prevent NSAID-induced neutrophil adherence to the vascular endothelium both in vivo (Asako *et al.* 1992a) and in vitro (Yoshida *et al.*, 1993). Evidence for a role for neutrophils in the pathogenesis of gastric ulceration in humans has

recently been provided (Taha *et al.* 1999). Patients taking NSAIDs who had significant numbers of neutrophils within the gastric mucosa were approximately six times more likely to develop an ulcer over the course of 24 weeks, than were patients who did not have significant numbers of neutrophils in their gastric mucosa. It is also noteworthy that, in a clinical trial examining the potential benefit of famotidine for the prevention of NSAID-related gastroduodenal ulcers, It was observed that an increased peripheral white blood cell count was a significant risk factor for ulcer development (Taha *et al.*, 1996). They suggested that this observation was consistent with the hypothesis that NSAID-induced gastropathy was mediated at least partially, by neutrophils.

How would the adherence of neutrophils to the vascular endothelium contribute to the formation of gastroduodenal ulcers? First, the adherence of neutrophils to the vascular endothelium is accompanied by an activation of these cells, leading to the release of proteases (e.g. elastase and collagenase) and oxygen-derived free radicals (e.g. superoxide anions). These substances may mediate much of the endothelial and epithelial injury caused by NSAIDs. This is supported by observations that the severity of NSAID-induced mucosal injury can be markedly diminished by compounds that scavenge oxygen-derived free radicals (Del Soldato *et al.*, 1985; Vaananen *et al.*, 1991), and by inhibitors of neutrophil-derived proteases (Yoshida *et al.*, 1995; Murakami *et al.*, 1999).

Secondly, neutrophil adherence to the endothelium, and the subsequent recruitment of other elements of blood (e.g. platelets), could produce an obstruction of the capillaries, thereby reducing gastric mucosal blood flow. In this respect, it should be noted that the well-characterized ability of NSAIDs to reduce gastric blood flow (Ashley *et al.*, 1985; Kitahora

and Guth, 1987; Gana *et al.*, 1987) has been shown to occur subsequent to the appearance of 'white thrombi' in the gastric microcirculation (Kitahora and Guth, 1987).

### 2.18.4 Inhibition of restitution

Damage to the gastric epithelium probably occurs on a daily basis but does not usually lead to deeper mucosal injury because of the ability of rapid (i.e. within minutes) repair that occur via the process of restitution. This process involves the rapid migration of healthy cells from the gastric pits to re-establish an intact epithelial barrier (Morris and Wallace, 1981). The cells move along the denuded basement membrane, which acts as a template and has been shown to be crucial to the restitution process (Morris and Wallace, 1981; Svanes *et al.*, 1982; Lacy and Ito, 1984; Moore *et al.*, 1989). The basement membrane can be damaged by acid leading to an inhibition of restitution and the progression of necrosis to deeper layers of the mucosa (Black *et al.*, 1985; Wallace and Whittle, 1986; Wallace and McKnight, 1990). This does not, however, occur in normal circumstances because of the formation over sites of injury (i.e. exposed basement membrane) of a microenvironment in which the pH is maintained at near neutral, even in the presence of a significant acid load in the lumen (Wallace and Whittle, 1986; Wallace and McKnight, 1990). A 'mucoid cap' consisting of mucus, cellular debris and plasma proteins (particularly fibrin) forms within seconds of gastric epithelial injury, trapping the plasma that leaks from the underlying microcirculation (Wallace and Whittle, 1986). It is this plasma which accounts for the near-neutral pH within the protective mucoid cap, since even a very brief cessation of mucosal blood flow results in a rapid decrease in the pH within the mucoid cap, which in turn results in the formation of haemorrhagic erosions (Wallace *et al.*, 1990).

As discussed above, NSAIDs can decrease mucosal blood flow. Thus, NSAIDs can cause gastric injury by interfering with the function of the mucoid cap and therefore the process of restitution. Following the systemic administration of an NSAID to an animal in which superficial epithelial injury had been induced, the pH within the mucoid cap that had formed over the sites of epithelial damage declines in parallel with the inhibition of prostaglandin synthesis (Wallace *et al.*,. 1990).Within minutes thereafter, the formation of haemorrhagic erosions becomes clearly evident. This effect could be prevented through the luminal delivery of exogenous prostaglandins, as could the formation of haemorrhagic erosions (Wallace *et al.*,. 1990).

**2.18.5 Repair of ulcers**

In addition to causing ulcer formation, NSAIDs can delay the healing of pre-existing ulcers and promote their bleeding (Stadler *et al.*, 1991; Armstrong and Blower, 1997). The effects on ulcer healing are probably related to the ability of NSAIDs to suppress prostaglandin synthesis. In normal gastric mucosa, prostaglandin synthesis occurs mainly via the cyclo-oxygenase-1 isoform. However, at a site of ulceration, and particularly around the ulcer margin, cyclo-oxygenase-2 appears to be the primary contributor to prostaglandin synthesis. Studies in mice and rats initially demonstrated the marked upregulation of cyclooxygenase-2 in ulcerated gastric tissue (Mizuno *et al.*, 1997; Schmassmann *et al.*, 1998). Moreover, the treatment of rats or mice with selective inhibitors of cyclooygenase-2 results in a significant delay of ulcer healing (Mizuno *et al.*, 1997; Schmassmann *et al.*, 1998). These observations, and reports of selective cyclooxygenase-2 inhibitors exacerbating intestinal inflammation and

ulceration (Reuter *et al*., 1996), suggest that caution should be exercised in regards to new cyclooxygenase-2 inhibitors as gastrointestinally safe (Wallace, 1999; Wallace *et al*., 1998). The ability of NSAIDs to promote the bleeding of pre-existing ulcers is most probably related to their inhibitory effects on platelet aggregation (Prichard *et al*., 1989; Hawkey *et al*., 1991). The inhibition of platelet aggregation by NSAIDs occurs as a consequence of the inhibition of thromboxane synthesis. Aspirin, unlike other NSAIDs produces an irreversible inhibition of thromboxane synthesis in the platelet. Thus, even the low doses of aspirin used for the prophylaxis of myocardial infarction and stroke can significantly increase the risk of gastrointestinal bleeding (The SALT Collaborative Group, 1991; Meade *et al*., 1992; Cryer *et al*., 1995; Agrawal, 1995).

### 2.18.6 Role of acid in ulcer formation

The observation that NSAID-induced ulcers can develop in achlorhydric individuals (Rashid and Tolfolon. 1993; Janssen *et al*. 1994), has contributed to a widely held belief that acid is not involved in the pathogenesis of these lesions. Further reinforcing this hypothesis are several studies demonstrating that treatment with histamine $H_2$-receptor antagonists did not reduce the incidence of NSAID-induced ulceration (Agrawal, 1995; Koch *et al*., 1995; Simon *et al*., 1994). However, many of these types of studies have demonstrated that $H_2$-antagonists and proton pump inhibitors can prevent NSAID-induced gastric lesions, but not the formation of the clinically more significant ulcers, as well as ulcer complications. It have been reported that a high dose of famotidine (40 mg twice daily) was effective in preventing NSAID-induced ulcers (Taha *et al*. 1999), and that omeprazole could significantly reduce the

incidence of NSAID-induced ulceration (Hawkey *et al.*, 1991). These studies suggested that a profound suppression of acid secretion, as is produced by omeprazole or by a high dose of famotidine, was necessary in order to have a significant impact on the incidence of NSAID-induced ulcers.

Acid may contribute to NSAID-induced ulcer formation in several ways. First, acid can exacerbate damage to the gastric mucosa induced by other agents. For example, acid can convert regions of ethanol-induced vascular congestion in the mucosa to actively bleeding erosions (Morris and Wallace, 1981). Secondly, acid will contribute to ulcer formation by interfering with haemostasis. Platelet aggregation, for example, is inhibited at a pH of less than 4 (Green *et al.*, 1978). Thirdly, as outlined above, acid can convert superficial injury to deeper mucosal necrosis by interfering with the process of restitution. Fourth, acid can inactivate several growth factors (e.g. fibroblast growth factor) that are important for the maintenance of mucosal integrity and for the repair of superficial injury, since these growth factors are acid-labile (Szabo *et al.*, 1994).

It is important to note that NSAIDs can increase gastric acid secretion, although it is not clear whether such effects have any impact on ulcer formation or healing. Prostaglandins exert inhibitory effects on parietal cells (Soll, 1986) so the inhibition of their synthesis by NSAIDs can result in an increase in gastric acid secretion (Ligumsky, Goto and Yamada, 1983).

## 2.19 EXPERIMENTAL WATER IMMERSION STRESS-INDUCED ULCER MODEL

Stress-induced ulcer model resembles the psychogenic factors in the pathogenesis of gastric ulcers in patients. Thus, stress-related animal experiments appear to be a human condition and

have allowed studies into pathogenic mechanisms as well as useful therapeutic interventions. Konturek *et al.*, (2003) reported that a stress-induced ulcer model which resembles human peptic ulcers both grossly and histopathologically, are useful in evaluating mucosal and cytoprotective drugs. Various physical and psychological stressors cause gastric ulceration in humans (Demirbilek *et al.*, 2004), and models have been developed to mimic the disease condition in humans. This model employs the restraint technique developed by Brodie and Hanson (Brodie and Hanson, 1960) coupled with the cold-water or ordinary-water immersion method by Levine (Levine, 1971). The combination of these methods is reported to be synergistic in inducing acute stress lesion in rats (Senay and Levine, 1967), arising mainly from physiological discomfort. Gastric ulcers induced by water-immersion stress (WIS) in rats or mice are known to resemble human peptic ulcers, both grossly and histopathologically (Konture *et al.*, 2003).The model is widely used and is reported to be useful for assessing or studying the effects of agents/medicines on the healing of ulcers in rats. Stress-induced ulcers manifest as single or multiple mucosal defects. The pathophysiology of stress-induced ulcers is complex. The ulcers are produced due to the release of histamine, leading to an increase in acid secretion, a reduction in mucus production (Kitagawa *et al.*,1979), pancreatic juice reflux, and poor flow of gastric blood (Guth, 1972).

Stress also causes an increase in gastrointestinal motility resulting in folds in the stomach (Peters and Richardson, 1983) that is more susceptible to damage when they come in contact with acid (Brodie and Hanson, 1960). Furthermore, stress has also been found to decrease the quality and amount of mucus adhering to the gastric mucosa. It has been suggested that, in conditions of emotional tension, there is not only a greater destruction of mucus and

decreased synthesis of its components, but also a quality change that affects the translation, acylation, and glycosylation of the ribosomal peptides (Peters and Richardson, 1983). Implicitly, the stomach wall mucus plays an important role in stress-induced glandular lesions. Increased vagal activity has also been reported to be one of the factors involved in stress-induced ulcers (Brodie and Hanson, 1960). Due to the critical role that mucus plays in protecting the stomach and also enhancing healing in the stomach walls, the model is recommended for use when evaluating mucosal and cytoprotective agents. The procedure for inducing ulcers with the water immersion stress-induced ulcer model, include animals being fasted for a period of 24-36 hours prior to the experiment. Ulcers are then induced by placing animals individually in a restricted cage and immersing them vertically in water tank, (15-20°C) gradually to the level of the xiphoid for 17 hours in the case of water-immersed model.

## 2.20 STARVATION-INDUCED ULCER MODEL

Starvation-induced ulceration was first reported by Singer in 1913 after observing lesions in the stomach of rats deprived food. Robert and Nezamis (1958) reported the presence of ulcers in the stomach of rats after four days of total starvation, and in the glandular portion following cortisone administration. Also Ogwa *et al.*, (1960) reported the production of gastric ulcers in the glandular portion of the stomach in mice after a period of starvation. Nutritional deprivation may result in degeneration and atrophy of gastric mucosal barriers, and in deficiency of precursors of mucosal cytoprotective substances, such as glutathione and

mucus. The pathological mechanisms underlying starvation-induced mucosal injury are complex. Starvation may enhance the activity of gastric mucosal offensive factors and/ or inhibit the activity of the defense system. Factors that are likely to be involved in the formation of starvation-induced ulcer include: increase in gastric acid, increase in generation of free radicals, reduction in mucosal cytoprotective substances, reduction in mucosal blood flow, and decrease in adenosine supply (Hung and Neu *et al.*, 1997). Reports also showed that there were acid back-diffusion, oxygen free radical generation and lowered mucus production correlated with ulcer formation in starved rats (Hung and Neu *et al.*, 1997).

## 2.2.1 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 follows:

| 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 of the method of Alphin and Wards (1968) to assess the ulceration produced by starvation and indomethacin-induction. They suggested that the method of scoring ulcer depends on the methods of induction of the ulcer since ulcerogenic 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.

## 2.22 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 and eradicating H. pylori.. 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. Also, over the next halfcentury,

therapeutic inhibition of acid secretion rested largely with the skilled hands of surgeons progressing from gastrectomy to highly selective vagotomy. Then, the synthesis of selective histamine, $H_2$ receptor antagonists not only provided the first effective and tolerated medication for acid inhibition but disproved the position taken by most of the researchers working on gastrin (Black *et al.*, 1972). Cimetidine established histamine as a prominent player in regulation of acid secretion in the human stomach (Black *et al.*, 1972).

### 2.22.1 Antacids

Neutralization of secreted acid with antacids are often used by patients for symptomatic relief of dyspepsia. The precise mechanisms by which antacids hasten the healing of peptic ulcerations are not clear, but a variety of cytoprotective effects have been proposed for these agents, especially those that contain aluminium (Konturek, 1993). The most commonly used agents are mixtures of aluminium hydroxide and magnesium hydroxide. Aluminum hydroxide can produce constipation and phosphate depletion. Magnesium hydroxide may cause loose stools. Calcium carbonate and sodium bicarbonate are potent antacids with varying levels of potential problems. The long-term use of calcium carbonate which converts to calcium chloride in the stomach can lead to the milk-alkali syndrome with hypercalcemia, hyperphosphatemia with possible renal calcinosis, and progression to renal insufficiency. Sodium bicarbonate may induce systemic alkalosis.

### 2.22.2 Histamine ($H_2$) Receptor Antagonists

Cimetidine was the first $H_2$ receptor antagonist used for the treatment of PUD. Now, four (cimetidine, ranitidine, famotidine, and nizatidine) of these agents are presently available. All

four agents are available over the counter without prescription in the United States. These compounds are competitive inhibitors of histamine-stimulated acid secretion. Famotidine also appears to have some component of non-competitive inhibition (Feldman and Burton, 1990). In addition to blocking histamine stimulated gastric acid secretion, all four agents suppress basal acid output as well as acid output stimulated by meals to comparable levels when used at therapeutic doses. The $H_2$-receptor antagonists are a remarkably safe and well-tolerated group of agents. Cimetidine has weak anti-androgenic activity that occasionally can cause gynecomastia and impotence (Chemer *et al.*, 1988). With short-term standard-dose therapy, these effects are rare. A variety of central nervous system symptoms have been reported rarely in patients taking $H_2$ receptor antagonists including headaches, restlessness, somnolence, dizziness, depression, memory problems, confusion, psychosis, and hallucinations. Myelosuppression is an uncommon, presumably idiosyncratic side effect of the $H_2$ receptor antagonists (Agura *et al.*, 1988). Cimetidine inhibits cytochrome P450.

### 2.22.3 Proton Pump Inhibitors (PPI)

The PPIs are a class of drugs that decrease gastric acid secretion through irreversible inhibition of $H^+,K^+$-ATPase, the proton pump of the parietal cell. Omeprazole, esomeprazole, lansoprazole, rabeprazole, and pantoprazole are five PPIs currently available. These agents are prodrugs that must be activated by acid to cause inhibition of $H^+,K^+$-ATPase. The half-life of PPIs is 18 hours. Thus, it can take between 2 and 5 days for gastric acid secretion to return to normal levels once these drugs have been discontinued. Mild to moderate hypergastrinemia has been observed in patients taking these drugs. Serum gastrin levels

return to normal levels within 1-2 weeks after drug cessation. Intrinsic factor production is also inhibited, but vitamin $B_{12}$-deficiency anaemia is uncommon, probably because of the large stores of the vitamin. As with any agent that leads to significant hypochlorhydria, PPIs may interfere with absorption of drugs such as ketoconazole, ampicillin, iron, and digoxin. Hepatic cytochrome P450 can be inhibited by the omeprazole and lansoprazole. Rabeprazole, pantoprazole, and esomeprazole do not appear to interact significantly with drugs metabolized by the cytochrome P450 system. PPIs provide superior acid suppression, healing rates, and symptom relief and are recommended as initial therapy for most patients. PPIs have been shown to provide earlier pain control and better healing rates at 4 weeks compared to $H_2$ blockers (85% versus 75%) (Poynard *et al.*, 1995). PPIs heal DUs in more than 95% of patients at 4 weeks and GUs in 80% to 90% of patients at 8 weeks (Vakil and Fennerty, 2003).

### 2.22.4 Helicobacter Pylori Eradication

Curing H. pylori infection not only heals peptic ulcer but also prevents ulcer relapse to <10-20% as compared to 59% in GU patients and 67% in DU patients when the organism is not eliminated (NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease, 1994). Multiple drugs including amoxicillin, metronidazole, tetracycline, clarithromycin, and bismuth compounds have been used for the therapy of H. pylori. The aim for initial eradication rates should be 85%-90%. No single agent is effective in eradicating the organism. Combination therapy for 14 days provides the greatest efficacy compared to 7-10 days regimens (Broutet *et al.*, 2003). Unfortunately, there is no universally regimen recommended for patients who have failed 2 courses of antibiotics.

### 2.22.5 Sucralfate

Sucralfate is a complex sucrose salt in which the hydroxyl groups have been substituted by aluminium hydroxide and sulfate. When exposed to gastric acid, the aluminum hydroxide dissociates, leaving sulfate anions that can bind electrostatically to positively charged proteins in damaged tissue. In this fashion, sucralfate adheres to ulcer craters, where it appears to form a protective barrier that may prevent further acid-peptic attack. Other proposed beneficial effects of sucralfate are enhancement of mucosal prostaglandin levels, stimulation of mucus and bicarbonate secretion, binding of bile salts, binding of epidermal growth factors, and promotion of angiogenesis (McCarthy, 1991). Toxicity from this drug is rare, with constipation being most common (2% - 3%).

### 2.22.6 Bismuth-Containing Preparations

Two colloidal preparations of bismuth have been most commonly used, colloidal bismuth subcitrate and bismuth subsalicylate. The mechanism by which these agents induce ulcer healing is unclear. Potential mechanisms include ulcer coating, prevention of further pepsin or hydrochloric-induced damage, binding of pepsin, and stimulation of prostaglandins, bicarbonate, and mucous secretion (Hall, 1989). These compounds are commonly used as one of the agents in an anti-H. pylori regimen as bismuth has documented antimicrobial actions against H. pylori. Adverse effects with short-term usage include black stools, constipation, and darkening of the tongue. Long-term usage with high doses, especially with the avidly absorbed colloidal bismuth subcitrate, may lead to neurotoxicity.

### 2.22.7 Prostaglandin Analogues

Misoprostol, a prostaglandin $E_1$ analogue, is the only prostaglandin analogue approved for the prevention of NSAID-induced ulcer disease. This drug not only enhances mucosal defense mechanisms but also inhibits gastric acid secretion. It has been shown that it significantly reduces nocturnal, basal, and meal stimulated acid secretion at a standard therapeutic dose, although the effect is not as potent as that of antisecretory agents (Davis, Fordtran and Dajani, 1988). The most common toxicity noted with this drug is diarrhoea (10%-30% incidence). Prostaglandins stimulate uterine smooth muscle. Uterine bleeding has been reported with prostaglandin analogs during the first trimester of pregnancy. It is therefore contraindicated in women who may be pregnant.

### 2.22.8 Treatment of NSAID/Aspirin Gastric or Duodenal Injury

Medical intervention for NSAID/aspirin-related mucosal injury includes treatment of an active ulcer and primary prevention of future injury. Current evidence indicates that PPIs are more effective than $H_2$ receptor antagonists and misoprostol in healing NSAID-associated ulcers when continuous NSAID/aspirin treatment is required. When NSAIDs can be discontinued, an $H_2$ receptor antagonist is an effective alternative. There are no data to support the substitution of conventional NSAIDs with cyclooxgenase-2 inhibitors in patients with active ulcers who continue to require anti-inflammatory therapy. Eradication of H. pylori and maintenance therapy with PPIs are effective in the prevention of aspirin-induced gastrointestinal lesions (NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease, 1994).

### 2.22.9 Surgery

Peptic ulcers are caused by imbalance between the gastroduodenal mucosal defensive factors such as bicarbonate and mucus versus aggressive factors like acid and pepsin. The pharmacotherapy of peptic ulcer has advanced a lot. The drug treatment of peptic ulcer has significantly brought down the morbidity and mortality and need for surgical interventions which may be attributed to the advent of $H_2$ blockers and proton pump inhibitors. Surgery is indicated in patients who are intolerant of medications or do not comply with medication regimes and in those at high risk of complications such as transplant recipients, patients dependent on steroids or NSAIDs, those with giant gastric or duodenal ulcer, and in those with ulcers that fail to heal with adequate treatment. Surgery should also be considered for patients who have a relapse during maintenance treatment or who have had multiple courses of medications (Palanivelu *et al.*, 2006). Truncal vagotomy, selective vagotomy, highly selective vagotomy, and partial gastrectomy are used in patients with DUs. Partial gastrectomy with gastroduodenal or gastrojejunal anastomosis may be used in patients with GUs.

## 2.23 PUD-RELATED COMPLICATIONS

Gastrointestinal bleeding is the most common complication observed in PUD. It occurs in 15% of patients and more often in individuals older than 60 years of age. The higher incidence in the elderly is likely due to the increased use of NSAIDs in this group (Hilton *et al.*, 2001). Up to 20% of patients with ulcer-related haemorrhages bleed without any preceding warning signs or symptoms. The second most common ulcer-related complication

is perforation. This complication occurs in 6%-7% of PUD patients (Valle, 2008). The incidence of perforation in the elderly is increased secondary to increased use of NSAIDs. Penetration is a form of perforation in which the ulcer bed tunnels into an adjacent organ. DUs tend to penetrate posteriorly into the pancreas, leading to pancreatitis. GUs tends to penetrate into the left hepatic lobe. Gastrocolic fistulas associated with GUs have also been described. Gastric outlet obstruction is the least common ulcer-related complication and occurs in 1%-2% of patients (Valle, 2008). Patients with recurrent duodenal or pyloric channel ulcers may develop pyloric stenosis as a result of acute inflammation, spasm, edema, or scarring and fibrosis.

## 2.24 PROSTAGLANDIN

The prostaglandins are a group of hormone-like lipid compounds that are derived enzymatically from fatty acids and have important functions in the animal body. Every prostaglandin contains 20 carbon atoms, including a 5-carbon ring. They are mediators and have a variety of strong physiological effects, such as regulating the contraction and relaxation of smooth muscle tissue. Prostaglandins are not endocrine hormones, but autocrine or paracrine, which are locally acting messenger molecules. They differ from hormones in that they are not produced at a specific site but in many places throughout the human body. Also, their target cells are present in the immediate vicinity of the site of their secretion (of which there are many). The prostaglandins, together with the thromboxanes and prostacyclins, form the prostanoid class of fatty acid derivatives, a subclass of eicosanoids (Ganong, 2001).

### 2.24.1 History and name

The name *prostaglandin* derives from the prostate gland. When prostaglandin was first isolated from seminal fluid in 1935 by the Swedish physiologist Euler, and independently by Goldblatt, it was believed to be part of the prostatic secretions. (In fact, prostaglandins are produced by the seminal vesicles). It was later shown that many other tissues secrete prostaglandins for various functions. The first total syntheses of prostaglandin F2α and prostaglandin E2 were reported by Corey in 1969, an achievement for which he was awarded the Japan Prize in 1989. In 1971, it was determined that aspirin-like drugs could inhibit the synthesis of prostaglandins. The biochemists Sune K. Bergstrom, Bengt I. Samuelsson and John R. Vane jointly received the 1982 Nobel Prize in Physiology or Medicine for their research on prostaglandins (Fabre *et al.*, 2001).

### 2.24.2 Biosynthesis

#### a. Biosynthesis of eicosanoids

Prostaglandins are found in most tissues and organs. They are produced by almost all nucleated cells. They are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. They are synthesized in the cell from the essential fatty acids (EFAs). An intermediate arachidonic acid is created from diacylglycerol via phospholipase-A2, then brought to either the cyclooxygenase pathway or the lipoxygenase pathway to form either prostaglandin and thromboxane or leukotriene respectively (figure 13). The cyclooxygenase pathway produces thromboxane, prostacyclin and prostaglandin D, E and F. Alternatively, the lipoxygenase enzyme pathway is active in leukocytes and in macrophages and synthesizes leukotrienes (Dubois, 2001, Gross *et al.*, 2007).

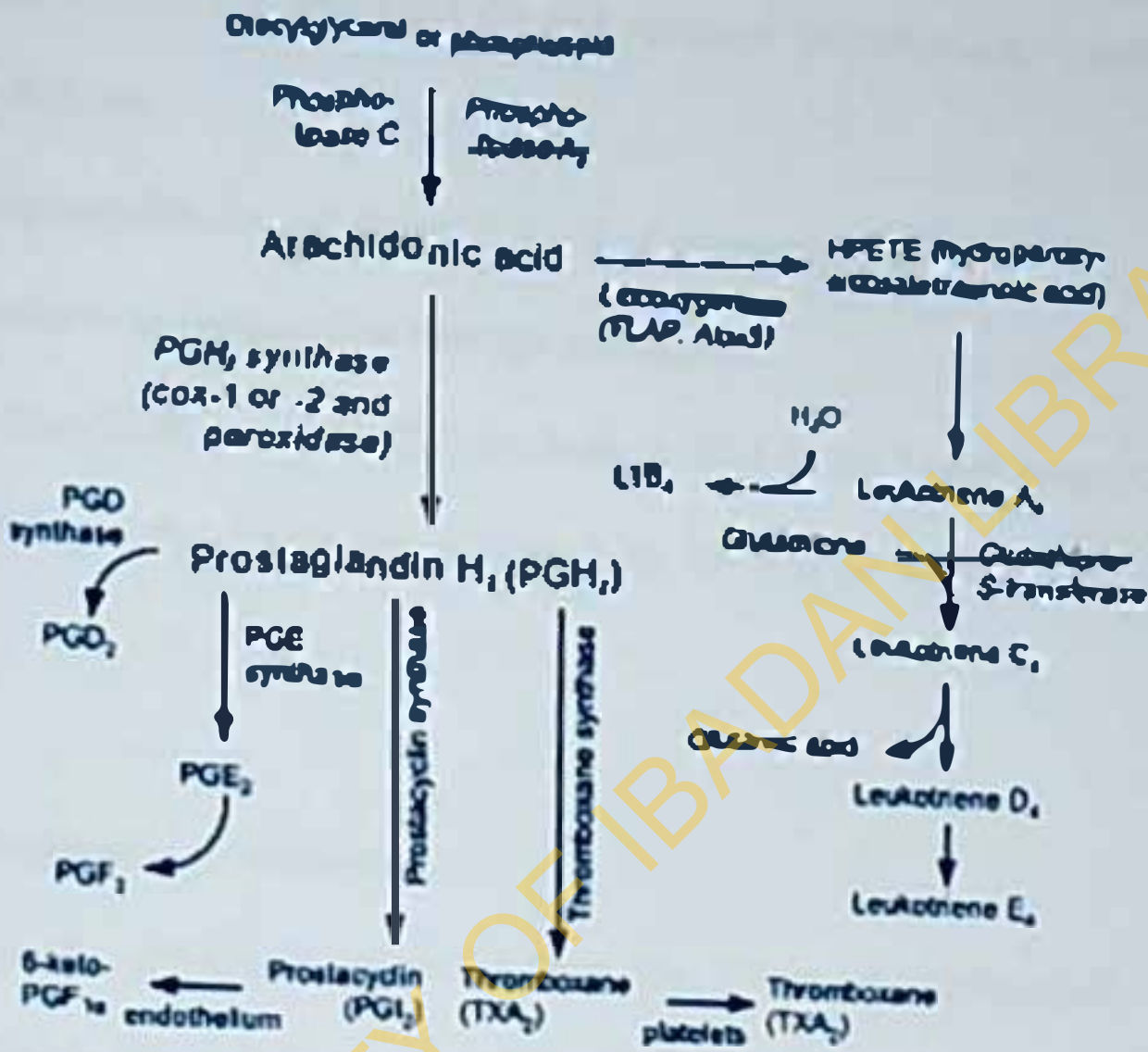



Figure 13: Diagram of biosynthesis of prostaglandins (Nicolaou and Sorensen, 1996)

### 2.24.3 Cyclooxygenases

Prostaglandins are produced following the sequential oxidation of AA, DGLA or EPA by cyclooxygenases (COX-1 and COX-2) and terminal prostaglandin synthases. The classic dogma is as follows:

- COX-1 is responsible for the baseline levels of prostaglandins.
- COX-2 produces prostaglandins through stimulation.

However, while COX-1 and COX-2 are both located in the blood vessels, stomach and the kidneys, prostaglandin levels are increased by COX-2 in scenarios of inflammation and growth.

### 2.24.4 Prostaglandin E synthase

Prostaglandin $E_2$ ($PGE_2$) is generated from the action of prostaglandin E synthases on prostaglandin $H_2$ ($PGH_2$). Several prostaglandin E synthases have been identified. To date, microsomal prostaglandin E synthase-1 emerges as a key enzyme in the formation of $PGE_2$.

### Terminal prostaglandin synthases

Terminal prostaglandin synthases have been identified that are responsible for the formation of other prostaglandins. For example, hematopoietic and lipocalin prostaglandin D synthases (hPGDS and lPGDS) are responsible for the formation of $PGD_2$ from $PGH_2$. Similarly, prostacyclin ($PGI_2$) synthase (PGIs) converts $PGH_2$ into $PGI_2$. A thromboxane synthase (TxAS) has also been identified. Prostaglandin-F synthase (PGFS) catalyzes the formation of 9α, 11β-$PGF_{2\alpha,\beta}$ from $PGD_2$ and $PGF_{2\alpha}$ from $PGH_2$ in the presence of NADPH. This enzyme

has recently been crystallized in complex with $PGD_2$ and bimatoprost (a synthetic analogue of $PGF_{2\alpha}$ (Fabre *et al.*, 2001; Ganong, 2001).

### 2.24.5 Functions of Prostaglandins

There are currently ten known prostaglandin receptors on various cell types. Prostaglandins ligate a sub-family of cell surface seven-transmembrane receptors, G-protein-coupled receptors. These receptors are termed $DP_{1,2}$, $EP_{1-4}$, FP, $IP_{1,2}$, and TP, corresponding to the receptor that ligates the corresponding prostaglandin (e.g., $DP_{1,2}$ receptors bind to $PGD_2$).

The diversity of receptors means that prostaglandins act on an array of cells and have a wide variety of effects such as:

- cause constriction or dilation in vascular smooth muscle cells
- cause aggregation or disaggregation of platelets
- sensitize spinal neurons to pain
- induce labour
- decrease intraocular pressure
- regulate inflammation
- regulate calcium movement
- regulate hormones
- control cell growth
- acts on thermoregulatory center of hypothalamus to produce fever
- acts on mesangial cells in the glomerulus of the kidney to increase glomerular filtration rate
- acts on parietal cells in the stomach wall to inhibit acid secretion
- brain masculinization (in rats).

Prostaglandins are potent but have a short half-life before being inactivated and excreted. Therefore, they send only paracrine (locally active) or autocrine (acting on the same cell from which it is synthesized) signals (Fabre *et al.*, 2001; Ganong, 2001, Gross *et al.*, 2007).

### 2.24.6 Types of Prostaglandins

The following is a comparison of different types of prostaglandin, prostacyclin $I_2$ ($PGI_2$), prostaglandin $E_2$ ($PGE_2$), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

**Type Receptor Receptor type Function**

**a. $PGI_2$ IP Gs**

- Vasodilation
- inhibit platelet aggregation
- Bronchodilation

**b. $PGE_2$**

**$EP_1$ Gq**

- Bronchoconstriction
- GI tract smooth muscle contraction

**$EP_2$ $G_s$**

- Bronchodilation
- GI tract smooth muscle relaxation
- Vasodilation

$EP_3$ Gi

- ↓ gastric acid secretion
- ↑ gastric mucus secretion
- uterus contraction (when pregnant)
- GI tract smooth muscle contraction
- Lipolysis inhibition
- ↑ autonomic neurotransmitters
- ↑ platelet response to their agonists and ↑ atherothrombosis in vivo (Fabre *et al.*, 2001).

c. $PGF_{1\alpha}$ FP Gq

- uterus contraction
- bronchoconstriction

## 2.24.7 Role in pharmacology

### Inhibition

Examples of prostaglandin antagonists are:

- NSAIDs (inhibit cyclooxygenase)
- Corticosteroids (inhibit phospholipase $A_2$ production)
- COX-2 selective inhibitors or coxibs
- Cyclopentenone prostaglandins may play a role in inhibiting inflammation

**Functions of the isoforms of COX**

The adherence of neutrophils to the vascular endothelium within the gastric microcirculation have been reported to contribute to the generation of mucosal injury by suppressing the tonic production of PGs such as prostacyclin (Wallace *et al.*, 1990, 1991, 1993) while prostaglandins derived from COX-2 have been reported to contribute to mucosal defense (Gretzer *et al.*, 1998). In other studies, Celecoxib have been reported to elicited significant leukocyte adherence in mesenteric vessels as compared to that obtained with indomethacin (McAdam *et al.*, 1999; Catella-Lawson *et al.*, 1999). Wallace *et al.*, 2000, in another study reported that COX-1 inhibition results in reduced gastric blood flow, whereas COX-2 inhibition leads to increased leukocyte adherence to the vascular endothelium. This may account for the gastric damage induced by the combination of SC-560 and Celecoxib. The authors also investigated the functional roles of COX isoforms in the gastric mucosa, showing that COX-1 dependent prostaglandins are involved in the maintenance of mucus/bicarbonate secretion and blood flow, while COX-2 protects the mucosa from leucocyte endothelial adhesion and supports epithelial renewal. Furthermore, COX-2 induced in ulcerated gastric mucosal is involved in the defense and repairing mechanisms of the mucosa and that its inhibition by a selective COX-2 inhibitor delays ulcer healing. In human stomach, COX-2 is exclusively expressed in gastric mesenchymal cells such as fibroblasts and in inflammatory cells of the ulcer bed and margins, suggesting that COX-2 expressed in mesenchymal cells at the ulcer margin plays a key role in the ulcer repair process (Miura et al., 2004).

### 2.24.8 Clinical uses

Synthetic prostaglandins are used:

- To induce childbirth (parturition) or abortion ($PGE_2$ or $PGF_2$, with or without mifepristone, a progesterone antagonist)
- To prevent closure of patent ductus arteriosus in newborns with particular cyanotic heart defects ($PGE_1$)
- To prevent and treat peptic ulcers (PGE)
- As a vasodilator in severe Raynaud's phenomenon or ischemia of a limb
- In pulmonary hypertension
- In treatment of glaucoma (as in bimatoprost ophthalmic solution, a synthetic prostamide analog with ocular hypotensive activity)
- To treat erectile dysfunction or in penile rehabilitation following surgery ($PGE_1$ as alprostadil).
- To treat egg binding in small birds
- As an ingredient in eyelash and eyebrow growth beauty products due to side effects associated with increased hair growth (Fabre *et al.*, 2001).

## 2.25 ANTIPSYCHOTICS

Antipsychotics also known as neuroleptics or major tranquilizers (Cubeddu *et al.*, 2009) are a class of psychiatric medication primarily used to manage psychosis (including delusions, hallucinations, or disordered thought), in particular in schizophrenia and bipolar disorder, and are increasingly being used in the management of non-psychotic disorders. From Moby's Medical Dictionary, the word neuroleptic originates from the Greek word "νεῦρον", neuron ("nerve") and lepsis ("seizure" or "fit").

## 2.25.1 History

The original antipsychotic drugs were discovered largely by chance and then tested for their effectiveness. The first, chlorpromazine, was developed as a surgical anaesthetic. It was first used on psychiatric patients because of its powerful calming effect; at the time it was regarded as a non-permanent "pharmacological lobotomy" (Pieters and Majerus, 2011). Lobotomy at the time was used to treat many behavioural disorders, including psychosis, although its effect was to markedly reduce behaviour and mental functioning of all types. However, chlorpromazine proved to reduce the effects of psychosis in a more effective and specific manner than lobotomy, even though it was known to be capable of causing severe sedation. The underlying neurochemistry involved has since been studied in detail, and subsequent antipsychotic drugs have been discovered by an approach that incorporates this sort of information.

The discovery of chlorpromazine's psychoactive effects in 1952 led to greatly reduced use of restraint, seclusion, and sedation in the management of agitated patients (Pieters and Majerus, 2011), and also led to further research that resulted in the development of antidepressants, anxiolytics, and the majority of other drugs now used in the management of psychiatric conditions. In 1952, Henri Laborit described chlorpromazine only as inducing indifference towards what was happening around them in non-psychotic, non-manic patients, and Jean Delay and Pierre Deniker described it as controlling manic or psychotic agitation. The former claimed to have discovered a treatment for agitation in anyone, and the latter team claimed to have discovered a treatment for psychotic illness (Healy, 2005). Until the 1970s there was considerable debate within psychiatry on the most appropriate term to use to describe the new

drugs (King and Voruganti, 2002). In the late 1950s the most widely used term was "neuroleptic", followed by "major tranquilizer" and then "ataraxic" (King and Voruganti, 2002). The first recorded use of the term tranquilizer dates from the early nineteenth century (Tranquillizer, 1989). In 1953, Yonkman, a chemist at the Swiss based Cibapharmaceutical company, fust used the term tranquilizer to differentiate reserpine from the older sedatives (Healy, 2008). The word neuroleptic was derived from the Greek: "νεῦρον"(neuron, originally meaning "sinew" but today referring to the nerves) and "λαμβάνω" (lambanō, meaning "take hold of"). Thus, the word means taking hold of one's nerves. This may refer to common side effects such as reduced activity in general, as well as lethargy and impaired motor control. Although these effects are unpleasant and in some cases harmful, they were at one time, along with akathisia, considered a reliable sign that the drug was working (Pieters and Majerus, 2011). The term "ataraxy" was coined by the neurologist Howard Fabing and the classicist Alister Cameron to describe the observed effect of psychic indifference and detachment in patients treated with chlorpromazine (Owens and Cunningham, 1999). This term derived from the Greek adjective "ἀτάραχτος" (ataraktos), means "not disturbed, not excited, without confusion, steady, calm" (King and Voruganti, 2002). In the use of the terms "tranquilizer" and "ataractic", medical practitioners distinguished between the "major tranquilizers" or "major ataractics", which referred to drugs used to treat psychoses, and the "minor tranquilizers" or "minor ataractics", which referred to drugs used to treat neuroses ((King and Voruganti, 2002). While popular during the 1950s, these terms are infrequently used today. They are being abandoned in favor of "antipsychotic", which refers to the drug's desired effects ((King and Voruganti, 2002). Today, "minor tranquilizer" can refer to anxiolytic and/or hypnotic drugs such as the benzodiazepines and nonbenzodiazepines, which

have some antipsychotic properties and are recommended for concurrent use with antipsychotics, and are useful for insomnia or drug-induced psychosis (Tasman, 1999). They are powerful (and potentially addictive) sedatives. Antipsychotics are broadly divided into two groups, the typical or first-generation antipsychotics and the atypical or second-generation antipsychotics. The typical antipsychotics are classified according to their chemical structure while the atypical antipsychotics are classified according to their pharmacological properties. These include dopamine antagonists and serotonin antagonists, multi-acting receptor-targeted antipsychotics (MARTA, those targeting several systems), and dopamine partial agonists, which are often categorized as atypical (Horacek *et al.*, 2006). First-generation antipsychotics, known as typical antipsychotics, were discovered in the 1950s. Most second-generation drugs, known as atypical antipsychotics, have been developed more recently, although the first atypical antipsychotic, clozapine, was discovered in the 1950s and introduced clinically in the 1970s. Both generations of medication tend to block receptors in the brain's dopamine pathways, but atypicals tend to act on serotonin receptors as well. The superiority of antipsychotics to placebo in the treatment of schizophrenia, bipolar disorder, and certain other psychiatric disorders is well-established, but their efficacy is suboptimal and their use is associated with important side effects, most notably movement disorders and weight gain (Frankenburg *et al.*, 2013).

### 2.25.2 Medical uses

Antipsychotics are most frequently used for the following conditions:

- Schizophrenia
- Schizoaffective disorder most commonly in conjunction with either an antidepressant

(in the case of the depressive subtype) or a mood stabiliser (in the case of the bipolar subtype).

- Bipolar disorder (acute mania and mixed episodes may be treated with either typical or atypical antipsychotics, although atypical antipsychotics are usually preferred.
- because they tend to have more favourable adverse effect profiles (Leucht *et al.*, 2009) and, according to a recent meta-analysis, they tend to have a lower liability for causing conversion from mania to depression (Goikolea *et al.*, 2013).
- Psychotic depression. It is a common practice for the attending psychiatrist to prescribe a combination of an atypical antipsychotic and an antidepressant as this practice is best supported by the evidence (Taylor *et al.*, 2012).
- Treatment-resistant (and not necessarily psychotic) major depression as an adjunct to standard antidepressant therapy (Taylor *et al.*, 2012).

**a. Schizophrenia**

Anti-psychotic drug treatment is a key component of schizophrenia treatment algorithms recommended by the National Institute of Health and Clinical Excellence (NICE), the American Psychiatric Association and the British Society for Psychopharmacology (Barnes, 2011). The main effect of treatment with antipsychotics is to reduce the so-called "positive" symptoms, including delusions and hallucinations. There is little evidence to support any significant impact of antipsychotic use on negative symptoms (such as apathy, lack of emotional affect, and lack of interest in social interactions) or on the cognitive symptoms (disordered thinking, reduced ability to plan and execute tasks) of schizophrenia (Miyamoto *et al.*, 2012).

Applications of antipsychotic drugs in the treatment of schizophrenia include prophylaxis in those showing symptoms that suggest that they are at high risk of developing psychosis, treatment of first episode psychosis, maintenance therapy, and treatment of recurrent episodes of acute psychosis (Barnes, 2011).

**b. Other uses of antipsychotics**

Besides the above uses, antipsychotics may be used for obsessive-compulsive disorder, posttraumatic stress disorder, personality disorders, Tourette syndrome, autism and agitation in those with dementia (Maher and Theodore, 2012). Risperidone may be useful for obsessive compulsive disorder (Maher and Theodore, 2012). The use of low doses of antipsychotics for insomnia, while common, is not recommended as there is little evidence of benefit and concerns regarding adverse effects (Maglione *et al.*, 2011; Coe and Hong, 2012). Low dose antipsychotics may also be used in treatment of impulse-behavioural and cognitive-perceptual symptoms of borderline personality disorder (American Psychiatric Association and American Psychiatric Association, 2001). In children they may be used in those with disruptive behavior disorders, mood disorders and pervasive developmental disorders or intellectual disability (Zuddas *et al.*, 2011). Antipsychotics are only weakly recommended for Tourette syndrome as well, though they are effective and side effects are common (Pringsheim *et al.*, 2012). The situation is similar for those on the autism spectrum (McPheeters *et al.*, 2011). Risperidone has been approved by the US FDA for the treatment of irritability in autistic children and adolescents (Posey *et al.*, 2008). Aggressive challenging behaviour in adults with intellectual disability is often treated with antipsychotic drugs despite lack of an evidence base.

### 2.25.3 Typical versus atypical Antipsychotics

While the atypical (second-generation) antipsychotics were marketed as offering greater efficacy and reduced side effects than typical medications this may not be true (Geddes *et al.*, 2000; Alexander *et al.*, 2011). One review concluded there were no differences (Horacek *et al.*, 2006) while another (Leucht *et al.*, 2003) found that atypicals were "only moderately more efficacious (Horacek *et al.*, 2006). These conclusions were, however, questioned by another review, which found that clozapine, amisulpride, and olanzapine and risperidone were more effective (Davis *et al.*, 2003; Horacek *et al.*, 2006). Many researchers question the first-line prescribing of atypicals over typicals, and some even question the distinction between the two classes (Owens, 2008; Fischer-Barnicol, *et al.*, 2008; Paczynski *et al.*, 2012). In contrast, other researchers point to the significantly higher risk of tardive dyskinesia and extrapyramidal symptoms with the typicals and for this reason alone recommend first-line treatment with the atypicals, notwithstanding a greater propensity for metabolic adverse effects in the latter (Casey, 1999; Meltzer and Bobo, 2006).

- First-generation (Typical antipsychotic)

- Butyrophenones

Benperidol (Anguil, Benguil, Frenactil, Glianimon)

- Bromperidol (Bromodol, Impromen)
- Droperidol (Droleptan, Inapsine)
- Haloperidol (Haldol, Serenace)
- Timiperone (Celmanil, Tolopelon)

### ii. Diphenylbutylpiperidine

- Fluspirilene (Imap)
- Penfluridol (Semap)

### iii. Phenothiazines

Acepromazine (Plegicil) - mostly used in veterinary medicine.

- Chlorpromazine (Largactil)
- Cyamemazine (Tercian)
- Dixyrazine (Esucos)
- Fluphenazine (Modecate)
- Levomepromazine (Levinan, Nozinan)
- Perazine (Peragal, Perazin, Pemazinum, Toxilan)
- Pericyazine (Neulactil, Neuleptil)
- Perphenazine (Trilafon)
- Pipotiazine (Lonseren, Piportil)
- Prochlorperazine (Compazine)
- Promethazine (Avomine)
- Prothipendyl (Dominal)
- Thioproperazine (Majeptil)
- Trifluoperazine (Stelazine)

### iv. Thioxanthenes

ChlorProthixene (Cloxan, Taractan, Truxal)

- Clopenthixol (Sordinol)
- Flupentixol (Depixol, Fluanxol)
- Tiotixene (Navane, Thixit)
- Zuclopenthixol (Acuphase, Cisordinol, Clopixol)(Baldessarini and Tarazi 2001).

v. Disputed/Unknown

This category is for drugs that have been called both first and second-generation, depending on the literature being used.

- Carpipramine (Defekton, Prazinil)
- Clocapramine (Clofekton, Padrasen)
- Molindone (Moban)
- Mosapramine (Cremin)
- Sulpiride (Meresa)
- Sultopride (Barnetil, Topral)
- Veralipride (Agreal) (Baldessarini and Tarazi 2001).

b. Second-generation (Atypical antipsychotic)

- Amisulpride (Solian)
- Amoxapine (Asendin)
- Aripiprazole (Abilify)
- Asenapine (Saphris, Sycrest)
- Clozapine (Clozaril)
- Blonanserin (Lonasen)

- Iloperidone (Fanapt, Fanapta, and previously known as Zomaril)
- Lurasidone (Latuda)
- Melperone (Buronil, Buronon, Eunerpan, Melpax, Neuril)
- Nemonapride (Emilace)
- Olanzapine (Zyprexa)
- Paliperidone (Invega)
- Perospirone (Lullan)
- Quetiapine (Seroquel)
- Remoxipride (Roxiam)
- Risperidone (Risperdal). Used off-label to treat Tourette syndrome and anxiety disorder.
- Sertindole (Serdolect, Serlect)
- Trimipramine (Surmontil)
- Ziprasidone (Geodon, Zeldox)
- Zotepine (Lodopin, Losizopilon, Nipolept, Setous) (Baldessarini and Tarazi 2001).

### 2.25.4 Mechanism of action of antipsychotic drugs

All antipsychotic drugs tend to block $D_2$ receptors in the dopamine pathways of the brain (Baldessarini and Tarazi 2001). This means that dopamine released in these pathways has less effect. Excess release of dopamine in the mesolimbic pathway has been linked to psychotic experiences. It has also been proven that less dopamine released in the prefrontal cortex in the brain, and excess dopamine released from all other pathways, has also been linked to psychotic experiences, caused by abnormal dopaminergic function as a result of patients

suffering from schizophrenia or bipolar disorder. Various neuroleptics such as haloperidol and chlorpromazine suppress dopamine throughout its pathways (Baldessarini and Tarazi 2001). In addition of the antagonistic effects of dopamine, antipsychotics (in particular atypical neuroleptics) also antagonize 5-$HT_{2A}$ receptors. Different alleles of the 5-$HT_{2A}$ receptor have been associated with schizophrenia and other psychoses, including depression (Schmidt *et al.*, 1995; McDonald and Murphy, 2003). Higher concentrations of 5-$HT_{2A}$ receptors in cortical and subcortical areas, in particular in the right caudate nucleus have been historically recorded (McDonald and Murphy, 2003). This is the same receptor that psychedelic drugs antagonize to various degrees, which explains the correlation between psychedelic drugs and schizophrenia (Amitabha, 2007). Typical antipsychotics are not particularly selective and also block dopamine receptors in the mesocortical pathway, tuberoinfundibular pathway, and the nigrostriatal pathway. Blocking $D_2$ receptors in these other pathways is thought to produce some unwanted side effects that the typical antipsychotics can produce (Baldessarini and Tarazi 2001. They were commonly classified on a spectrum of low potency to high potency, where potency referred to the ability of the drug to bind to dopamine receptors, and not to the effectiveness of the drug. High-potency antipsychotics such as haloperidol, in general, have doses of a few milligrams and cause less sleepiness and calming effects than low-potency antipsychotics such as chlorpromazine and thioridazine, which have dosages of several hundred milligrams. The latter have a greater degree of anticholinergic and antihistaminergic activity, which can counteract dopamine-related side-effects (Baldessarini and Tarazi 2001).

Atypical antipsychotic drugs have a similar blocking effect on $D_2$ receptors, however, most

also act on serotonin receptors, especially 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors. Both clozapine and quetiapine appear to bind just long enough to elicit antipsychotic effects but not long enough to induce extrapyramidal side effects and prolactin hypersecretion (Stahl, 2003). 5-$HT_{2A}$ antagonism increases dopaminergic activity in the nigrostriatal pathway, leading to a lowered extrapyramidal side effect liability among the atypical antipsychotics (Stahl, 2003; Gross and Geyer, 2012).

### 2.25.5 Antipsychotic Formulations

Antipsychotics are sometimes administered as part of compulsory psychiatric treatment via inpatient (hospital) commitment or outpatient commitment. They may be administered orally or, in some cases, through long-acting (depot) injections.

### 2.25.6 Adverse effects

Antipsychotics are associated with a range of side effects. It is well-recognized that many people stop taking those (around two-thirds even in controlled drug trials) due in part to adverse effects (Bellack, 2006). Common (≥ 1% and up to 50% incidence for most antipsychotic drugs) adverse effects of antipsychotics include

- Sedation (particularly common in patients on clozapine, olanzapine, quetiapine, chlorpromazine and zotepine (Leucht *et al.*, 2013).
- Headaches
- Dizziness
- Diarrhea
- Anxiety

- Extrapyramidal side effects (particularly common in patients on first-generation antipsychotics), which includes:

  - Akathisia — an often distressing sense of inner restlessness.
  - Dystonia
  - Parkinsonism
  - Tremor

- Hyperprolactinaemia (rare for those on clozapine, quetiapine and aripiprazole (Taylor *et al.*, 2012; Leucht *et al.*, 2013), which can cause:

- Galactorrhoea — unusual secretion of breast milk, Gynaecomastia, Sexual dysfunction (in both sexes) and Osteoporosis

- Orthostatic hypotension
- Weight gain (particularly prominent in patients on clozapine, olanzapine, quetiapine and zotepine (Leucht *et al.*, 2013).
- Anticholinergic side-effects (common for olanzapine, clozapine; less likely on risperidone (Lieberman, 2004).

Rare/Uncommon (<1% incidence for most antipsychotic drugs) adverse effects of antipsychotics include

- Blood dyscrasias (e.g., agranulocytosis, leukopaenia, and neutropaenia), which is more common in patients on clozapine.

- Metabolic syndrome and other metabolic problems such as Type II diabetes mellitus — particularly common with clozapine, olanzapine and zotepine. Evidence suggests that females are more sensitive to the metabolic side effects of first-generation antipsychotic drugs than males (Weston-Green *et al*., 2010).

- Pancreatitis (Koller *et al*., 2003)
- QT interval prolongation — more prominent in patients on amisulpride, pimozide, sertindole, thioridazine and ziprasidone (Taylor *et al*., 2012; Leucht *et al*., 2013).
- Seizures, which is particularly common in patients on chlorpromazine and clozapine.
- Thrombocmbolism
- Myocardial infarction
- Stroke

Some studies have found decreased life expectancy associated with the use of antipsychotics, and argued that more studies are needed (Joukaman *et al*., 2006; Weinmann *et al*., 2009). Antipsychotics may also increase the risk of early death in individuals with dementia (American Geriatrics Society updated Beers Criteria for potentially inappropriate medication use in older adults, 2012). In individuals without psychosis, doses of antipsychotics can produce the "negative symptoms" of schizophrenia such as amotivation (Artaloytia *et al*., 2006). Antipsychotics typically worsen symptoms in people who suffer from depersonalisation disorder (Medford *et al*., 2005).

**a. Other adverse effects of antipsychotics**

Loss of grey matter and other brain structural changes over time are observed in

schizophrenia. Meta analyses of the effects of antipsychotic treatment on the course of grey matter loss and structural changes have reached conflicting conclusions. A meta- analysis conducted in 2012 made a conclusion that grey matter loss is greater in patients treated with first generation antipsychotics compared to those treated with atypicals, and hypothesized a protective effect of atypicals as one possible explanation (Vita *et al.*, 2012). A second meta-analysis suggested that treatment with antipsychotics was associated with increased grey matter loss (Radua *et al.*, 2012).

## 2.26 RISPERIDONE

Risperidone is an antipsychotic drug having anxiolytic activity that is used to treat schizophrenia (including adolescent schizophrenia), schizoaffective disorder, the mixed and manic states of bipolar disorder, and irritability in people with autism. It is also used to treat delusional psychosis and psychotic depression (Ishida-Tokuda *et al.*, 1996). Risperidone is a second-generation atypical antipsychotic (figure 14). It is a dopamine antagonist possessing anti-serotonergic, anti-adrenergic and anti-histaminergic properties. Adverse effects of risperidone include significant weight gain and metabolic problems such as diabetes mellitus type 2, as well as tardive dyskinesia and neuroleptic malignant syndrome. Risperidone and other antipsychotics also increase the risk of death in people with dementia.

The drug was developed by Janssen-Cilag, subsidiary of Johnson & Johnson, from 1988-1992 as an improvement from the typical antipsychotic and first approved by the FDA in 1994 (http:// web. archive. org/ web, 1994). It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.

Risperidone undergoes hepatic metabolism and renal excretion. Risperidone has been classified as a "qualitatively atypical" antipsychotic agent with a relatively low incidence of extrapyramidal side effects (when given at low doses) that has more pronounced serotonin antagonism than dopamine antagonism. It has actions at several 5-HT (serotonin) receptor subtypes. These are $5\text{-}HT_{2C}$, linked to weight gain, $5\text{-}HT_{2A}$, linked to its antipsychotic action and relief of some of the extrapyramidal side effects experienced with the typical neuroleptics (Brunton *et al.*, 2010). They found that D-amino acid oxidase, the enzyme that catalyses the breakdown of D-amino acids (e.g. D-alanine and D-serine — the neurotransmitters) is inhibited by risperidone.

2.26.1 Risperidone acts on the following Receptors

a. Dopamine receptors

This drug is an antagonist of the $D_1$ ($D_1$, and $D_5$) as well as the $D_2$ family ($D_2$, $D_3$ and $D_4$) receptors. This drug has "tight binding" properties, which means it has a long half-life and like other antipsychotics, risperidone blocks the mesolimbic pathway, the prefrontal cortex limbic pathway, and the tuberoinfundibular pathway in the central nervous system. Risperidone may induce extrapyramidal side effects, akathisia and tremors, associated with diminished dopaminergic activity in the striatum.

b. Serotonin receptors

Its action at these receptors may be responsible for its lower extrapyramidal side effect liability (via the $5\text{-}HT_{2A/C}$ receptors) and improved negative symptom control compared to typical antipsychotics such as haloperidol for instance. Its antagonistic actions at the $5\text{-}HT_{2C}$ receptor may account, in part, for its weight gain liability.

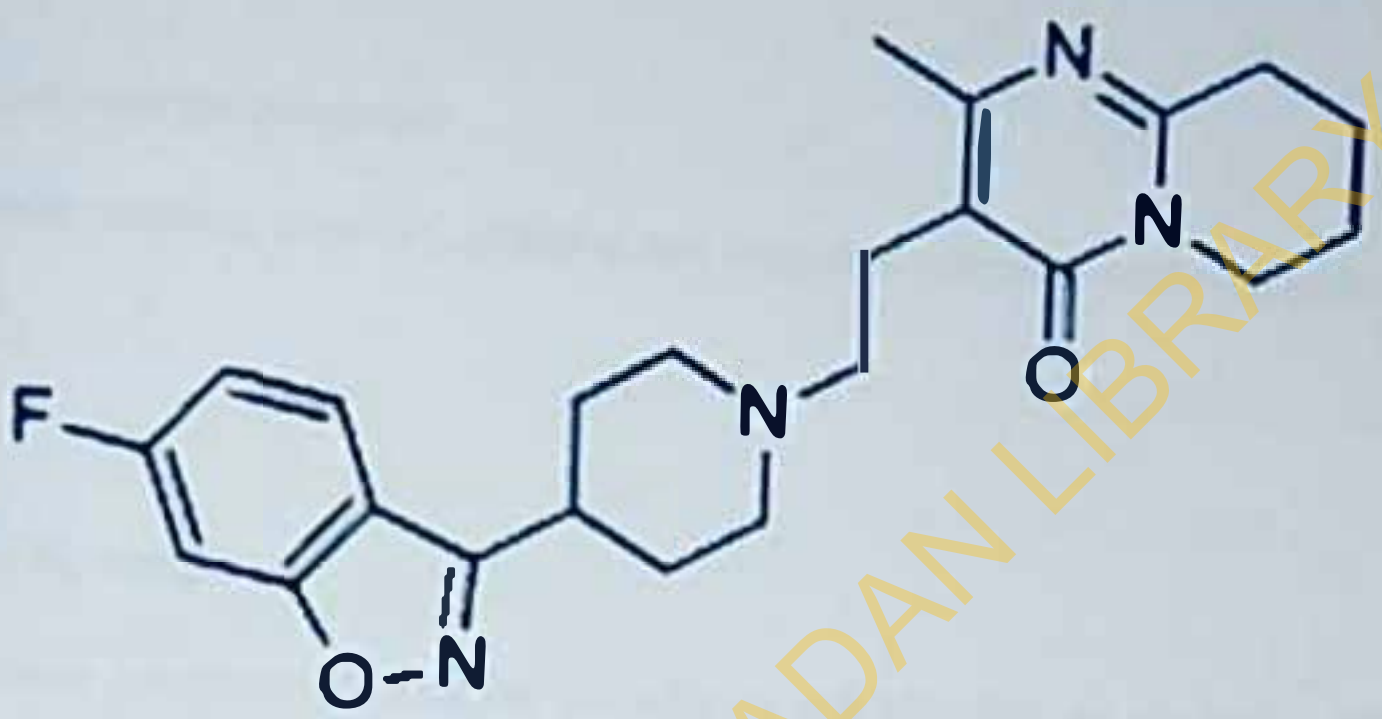



Figure 14. Chemical Structure of risperidone (Baldessarini and Tarazi, 2001).

**c. Alpha $\alpha_1$ adrenergic receptors**

This action accounts for its orthostatic hypotensive effects and perhaps some of the sedating effects of risperidone.

**d. Alpha $\alpha_2$ adrenergic receptors**

Perhaps greater positive, negative, affective and cognitive symptom control (Hecht and Landy, 2012).

**e. Histamine $H_1$ receptors**

Effects on these receptors account for its sedation and reduction in vigilance. Though this medication possesses similar effects to other typical and atypical antipsychotics, it does not possess an affinity for the muscarinic acetylcholine receptors. In many respects, this medication can be useful as "acetylcholine release-promoter" similar to gastrointestinal drugs such as metoclopramide and cisapride. $H_2$ receptor antagonists and proton-pump inhibitors are currently used anti-ulcer drugs.

# CHAPTER THREE

## 3.1 MATERIALS AND METHODS

Feeds, Sensitive weighing balance, Dissecting set, Animal cages, Laboratory glassware, Cotton wool, Plain specimen bottles, Petri dishes, Syringes, Wister rats, Spectrophotometer (Perkin Elmer UV visible spectrophotometer Lambda 3B), Operating table, beakers, Conical flasks, Water bath, Olympus optical microscope, Homogenizer, modified Langerdoff apparatus.

## 3.2 CHEMICALS

### 3.2.1 Drugs/Chemicals/Reagents

Risperidone (Jiangsu Suzhong Haixin Pharm CO, China), Histamine acid phosphate (Sigma-Aldrich, St Louis MO), Ketamine Hydrochloride (Rotexmedica, Trittau, Germany), Carbamoylcholine chloride (Carbacol: Sigma- Aldrich MO), Pentagastrin (Sigma Aldrich), Zinc Chloride (Sigma- St Louis, MO), Indomethacin (MSD), SC- 560 (Santa Cruz Biotechnology, USA) and Celebrex (Celecoxib Pfizer, Germany), Trichloroacetic acid, Magnesium chloride (Sigma- St Louis, MO), Sodium chloride (Sigma- St Louis, MO), Hydrochloric acid (Sigma. St Louis, MO), Thiobarbituric acid (Sigma- St Louis, MO), Alcian blue, Sodium acetate(Sigma- St Louis, MO), Diethyl ether (Sigma- St Louis, MO), Sodium hydroxide (Sigma- St Louis, MO). Chemicals and reagents were of analytical grade.

## 3.3 SOLUTIONS

Distilled water, 0.9% 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, histamine acid phosphate, 1% phenolphthalein solution, 40 mg/ml indomethacin, pentagastrin, carbachol.

### 3.3.1 Preparation of Stock Solutions and Reagents

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

(b) 0.1M Alcian blue solution: The solution was prepared by first dissolving 0.1g of Alcian blue in 100 ml of distilled water, and the resultant solution was dissolved in 0.16M sucrose solution. This was 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 100 ml of distilled water.

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

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

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

(h) 5 mg/ml indomethacin: It was prepared by dissolving 25 mg of the drug in 5 ml 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 100 ml of distilled water. From this stock solution, 1ml was taken and made up to 400 ml 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 50 ml of absolute alcohol. After the dissolving of the salt, 50 ml of distilled water was added to the solution.

## 3.4 CHOICE OF ANIMALS

A total of three hundred and thirty-two (336) adult male Wister rats of weights between 180-210 grams were used for this work. The choice of male rats was to maintain a fairly constant physiological condition, as gastric acid secretion does vary with oestrous cycle in female rats (Amuie and Omole, 1970). The animals were purchased from the Central animal house, College of Medicine, University of Ibadan, Nigeria and housed in a clean, well-ventilated room (25°C) 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 study was conducted in accordance with the Organization for Economic Development (OECD) guidelines on good laboratory practice (2001) and the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed.

## 3.5 PRETREATMENT OF ANIMALS AND GROUPINGS

Risperidone was administered orally at various doses as reported by Saxena and Singh, (2011) for 21 days. Six different studies were carried out and each study has four (4) sub-groups of eight (8) animals as listed below:

Group 1 = Distilled water (Control)

Group 2 = risperidone (0.1mg/kg)

Group 3 = risperidone (0.3mg/kg)

Group 4 = risperidone (0.5mg/kg)

## 3.6 EXPERIMENTAL DESIGN

The work was designed to evaluate the anti-gastric ulcer effect of risperidone and its mechanisms of action and was divided into six major studies;

1. To investigate the acclaimed anti-ulcer effect of risperidone using different ulcer models; Water immersion-restraint stress (WIRS) - , Indomethacin- and Starvation- induced gastric ulcer.

2. To determine the effect of risperidone on basal and maximal gastric acid secretion using three agonists namely histamine, pentagastrin (gastrin) and carbachol (acetylcholine).

3. To examine the gastroprotective activity of risperidone by determining its effect on gastric mucus secretion and gastric mucus cells count.

4. To determine the effect of risperidone on indomethacin, cyclooxygenase inhibitors (SC - 560 and Celecoxib) induced gastric ulceration.

5. To determine the antioxidant status of risperidone on treated animals by measuring malondialdehyde (MDA) concentration.

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

## 3.7 ANIMAL GROUPINGS, TREATMENT, AND PROCEDURES

### 3.7.1 Effect of risperidone on induced gastric ulceration

A total of thirty two rats were used for each ulcer study model. The animals in each study model were divided into four sub-groups of eight rats and treated for 21 days orally on a daily basis. Group 1 was the control and was treated with distilled water. Groups 2, 3 and 4 were treated with risperidone doses of 0.1mg/kg, 0.3 mg/kg, 0.5 mg/kg orally respectively (Saxena and Singh, 2011). After the treatment period the gastric ulceration studies were carried out separately. Three experimental ulcer models were used:

a. Water immersion restraint stress (WIRS) -induced ulcers

b. Indomethacin - induced ulcers

c. Starvation - induced ulcers.

#### a. Water immersion restraint stress-induced (WIRS) ulcer

After the treatment period, the animals were fasted for 24 hours and each placed in a restraint device. The animal was immersed up to its xiphoid process in a 22°C water bath for 17 hours

(Byun *et al.*, 2007). After 17 hours, the animals were sacrificed by cervical dislocation. Their stomachs were removed, opened by cutting along the whole length of the greater curvature, turned inside out and then pinned to a cork mat. This was moistened with normal saline to prevent autolysis. Macroscopic examinations of the washed stomachs were carried out using a magnifying hand lens while assessment of gastric mucosal lesion was carried out according to the method of Desai *et al.*, 1999 as stated below:

**Scoring Method used:**

| Ulcer score | | Criteria |
|---|---|---|
| 0 | - | no ulcer |
| 1 | - | Superficial mucosal erosion |
| 2 | - | Deep ulcer or transmural necrosis |
| 3 | - | Perforated or penetrated ulcer |

**b. Indomethacin- induced gastric ulceration**

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). Indomethacin at 40 mg/kg BW (Merck, Sharp & Dobme, 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 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 3 mm in diameter

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

where n = number of rats

c. Starvation – induced ulceration

The animals were deprived of food for 6 days within the 21 days pretreatment period but had access to water *ad libitum*. On completion of 6 days food deprivation, the animals were sacrificed by cervical dislocation. Their stomachs were removed, opened by cutting along the whole length of the greater curvature, turned inside out and then pinned to a cork mat. This was moistened with normal saline to prevent autolysis. The method used for ulcer assessment of the degree of gastric ulceration was that of 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:

Scoring procedures for Starvation - induced ulcers

| Ulcer score | Criteria |
|---|---|
| 0 - | Normal stomach, no ulceration |
| 1 - | Focal ulceration of part or whole of mucosal surface not more than one lesion seen. |
| 2 - | Ulcers occur frequently |
| 3 - | Subtotal necrosis and ulceration where only muscularis external was spared |
| 4 - | Necrosis with actual perforation. |

[Preliminary study had earlier showed measurable ulcer lesions occurring on day 5 while some animals died by day 7 of starvation. However, no recorded death on day 6 ].

### 3.7.2 Effect of risperidone on basal and histamine-induced gastric acid secretion.

The basal acid secretion and the maximal acid secretion were measured. A total of thirty two rats were used for this study. They were divided into four groups of eight rats and treated for 21 days orally on a daily basis. Group 1 was the control and was treated with distilled water. Group 2, Group 3 and Group 4 were treated with risperidone at doses of 0.1 mg/kg, 0.3 mg/kg, 0.5 mg/kg orally respectively (Saxena and Singh, 2011).

**a. Preparation of anaesthesia**

Urethane granules were obtained from the Department of Physiology of the University of Ibadan. 25 g was weighed out and dissolved in 100 ml of distilled water to give a concentration of 25% w/v urethane.

$$\text{Concentration} = \frac{\text{mass}}{\text{Volume}} = \frac{25\text{ g}}{100\text{ ml}} = 0.25\text{ g/ml} = 0.25 \times 1000 = 250\text{ mg/ml}$$

$$\text{The volume of solution to be administered} = \frac{\text{Dosage value}}{\text{Concentration}}$$

$$= \frac{150\text{ mg/100g}}{250\text{ mg/ml}}$$

$$= 0.6\text{ ml/100g.}$$

Therefore, 0.6 ml of 0.25g/ml of urethane was administered per 100g weight of an animal.

**b. Preparation and the dosage value of histamine**

A stock solution of histamine with concentration of 20 mg/ml was constituted from the laboratory of the Department of Physiology, University of Ibadan. The required dosage earlier reported was 50 mg/kg (Thompson *et al*, 1967).

Calculating,

1 ml from the stock solution was mixed with 9 ml of distilled water to give 2 mg/ml

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

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

The volume of the above solution to be given = $\frac{\text{Dosage value}}{\text{Concentration}}$

$= \frac{5\ \text{mg/100g}}{2\ \text{mg/ml}}$

$= 2.5\ \text{ml/100g}.$

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

c. Surgerical procedure for GAS collection

The animals were tied to the dissecting board after anaesthesia. An incision 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 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 incision was made an inch distal to the pyloro-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. 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 from the toe-ward direction and tied. After cannulating the femoral vein, the rate of flow of the perfusing fluid from the modified 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 Ghosh and Schild (1958). The collected effluent was titrated with 0.0025N NaOH after adding two drops of phenolphthalein to it.

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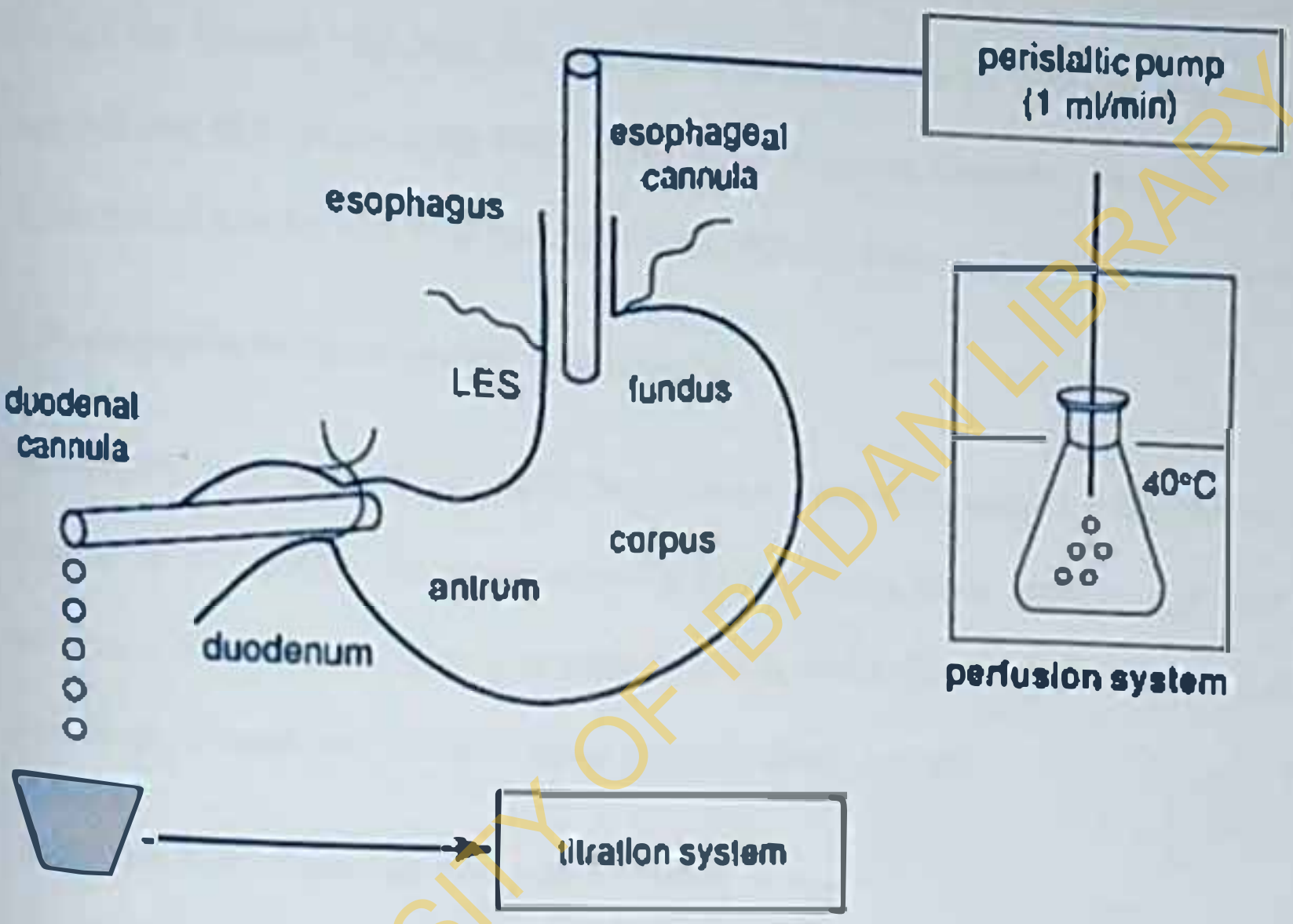


Figure 15 Illustration of the rat stomach preparation.

d. Maximum Secretion

Collection of Maximal Gastric Acid Secretion using different secretagogues:

i. Histamine-induced gastric acid secretion

A dose of 2.5 ml/100g body weight histamine acid phosphate was injected intravenously (i.v.) through the femoral vein into the rats after anaesthetized with urethane according to their body weights (0.6 ml per 100g BW). Then 10ml of gastric contents was collected at the rate of 1ml per minute for one hour twenty minutes (80 minutes) using gastroduodenal cannula.

ii. Pentagastrin-induced gastric acid secretion

Pentagastrin at a dose of 25 µg/kg body weight was administered intraperitoneally (i.p.) (Fatema *et al.*, 2006). Ketamine chloride (0.2 ml/100g body weight), *i.p.* was used as anesthesia. Then 10ml of gastric contents was collected at the rate of 1ml per minute for one hour twenty minutes (80 minutes) using gastroduodenal cannula.

iii. Carbachol-Induced gastric acid secretion

Carbachol at a dose of 4 µg/kg body weight was administered intraperitoneally (i.p.) (Naseri *et al.*, 2007). Ketamine chloride (0.2 ml/100g body weight), *i.p.* was used as anesthesia. Then 10ml of gastric contents was collected after 15 minutes, after which the timing for 10ml collection was made every 10 minutes for one hour twenty minutes (80 minutes).

e. Measurement of gastric acid concentration in samples

Titrations and calculations using the principle of volumetric analysis were used to measure the strength of the samples collected i.e basal and maximal gastric secretions.

**f. 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 from the burette reading.

**g. Volumetric Analysis**

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

$$M_A V_A = M_B V_B \quad \ldots\ldots (1)$$

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

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

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

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

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

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

But since M/400 NaOH was used = $M_o = 1/400$ M

Substituting for Mo

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

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

But 10ml of acid was used foreach titration i.e $V_A = 10$ml

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

Therefore, $0.25V_B$ represents concentration of the acid in effluent in meq/litre.

### 3.7.3 Effect of risperidone on gastric mucus secretion

A total of thirty two rats were used for this study. They were divided into four sub-groups of eight rats and treated for 21 days orally with risperidone. Group 1 was the control and was treated with distilled water. Groups 2, 3 and 4 were given risperidone at doses 0.1mg/kg, 0.3 mg/kg and 0.5 mg/kg respectively (Saxena and Singh, 2011). The rats were sacrificed by cervical dislocation and their stomachs removed and weighed. The glandular portion of each stomach was opened along the lesser curvature. The everted stomachs were soaked for two hours in 0.1% Alcian blue dissolved in 0.16M sucrose buffered with 0.05M sodium acetate, adjusted to pH 5.8 with hydrochloric acid. Uncomplexed dye was removed by two successive washes at 15 ond 45 minutes in 0.25M sucrose. Dye complexed with mucus was diluted by immersion in 10ml aliquots of 0.5M Magnesium Chloride for 2 hours. The resulting blue solutions were shaken briefly with equal volume of diethyl ether and absorbance of aqueous phase was measured at 605nm using spectrophotometer (Corney *et al.*, 1974).

The absorbance of each solution was then used to calculate the various concentrations of dye and the weight of dye (expressed in mg) deduced, using a standard curve. The weight of dye was then expressed over the weight of the stomach, to give the weight of mucus secreted.

Protedures for measurement were that described by Corney *et al* (1974).

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

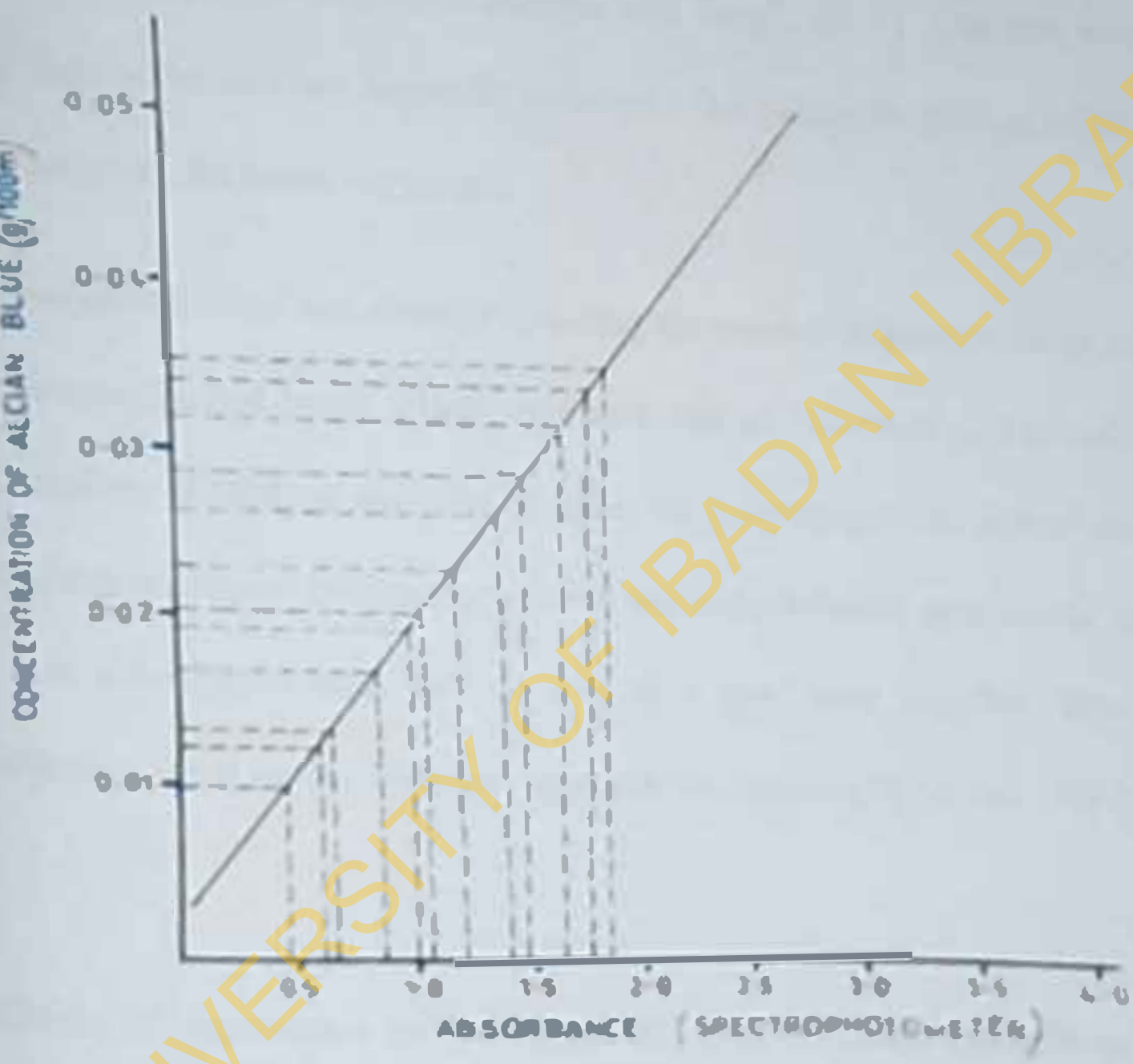


Figure 16: Standard curve of Alcian Blue dye (Concentration of dye against Absorbance)

### 3.7.4 Effect of risperidone on gastric mucus cell count

A total of thirty two rats were used for this study. They were divided into four sub-groups of eight rats and treated for 21 days orally with risperidone. Group 1 was the control and was treated with distilled water. Groups 2, 3 and 4 were given risperidone at doses 0.1mg/kg, 0.3 mg/kg and 0.5 mg/kg respectively (Saxena and Singh, 2011). The rats were sacrificed by cervical dislocation and the stomachs removed. 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 stained with Haematoxylin and Eosin. These were 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 1 $mm^2$ were assessed. This method is an improvement over the earlier described approach for counting by Li *et al* (2002).

### 3.7.5 Effects of risperidone on indomethacin, cyclooxygenase inhibitors: SC 560 and celecoxib induced gastric ulceration

A total of thirty two rats were used for this study. They were divided into four sub-groups of eight rats and treated for 21 days orally with risperidone. Group 1 was the control and was treated with distilled water. Groups 2, Group 3 and Group 4 were given risperidone at doses 0.1mg/kg, 0.3 mg/kg and 0.5 mg/kg respectively (Saxena and Singh, 2011). The same procedure for indomethacin -induced gastric ulceration was adopted with the following

Control: Indomethacin- 40 mg/kg (Elegbe and Bamgbose, 1976)

Group 1: SC 560 - 40mg/kg (Wallace *et al.*; 2000)

Group 2: Celecoxib - 15mg/kg (Wallace *et al.*; 2000)

Group 3: SC-560 (40mg/kg) and celecoxib (15mg/kg) combination (Wallace *et al.*; 2000)

Group 4: Risperidone (0.5mg/kg) + SC-560 (40mg/kg) + celecoxib (15mg/kg)

The COX inhibitors SC 560 and celecoxib were administered orally 4 hours before the animals were sacrificed.

### 3.7.6 Effect of risperidone on Malondialdehyde Concentration

A total of thirty two rats were used for this study. They were divided into four sub-groups of eight rats and treated for 21 days orally with risperidone. Group 1 was the control and was treated with distilled water. Groups 2, 3 and 4 were given risperidone at doses 0.1mg/kg, 0.3 mg/kg and 0.5 mg/kg respectively (Saxena and Singh, 2011). Lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) produced according to the method of Gutteridge and Wilkins (1982).

**Principle**

This method is based on the reaction between 2-thiobarbituric acid (TBA) and malonialdehyde (MDA) which is an end- product of lipid peroxides during 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 $1.56 \times 10^5 M^{-1}cm^{-1}$ according to the expression of Adam Vizi and Seregi (1982).

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

### 3.7.7 Effect of risperidone on the histological changes of the gastric mucosal.

A total of thirty two rats were used for this study. They were divided into four sub-groups of eight rats and treated for 21 days orally with risperidone. Group I was the control and was treated with distilled water. Groups 2, 3 and 4 were given risperidone at doses 0.1mg/kg, 0.3 mg/kg and 0.5 mg/kg respectively (Saxena and Singh, 2011). After the 21 days treatment period the rats were sacrificed by cervical dislocation, the stomachs 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 (H&E) and periodic acid Schiff (PAS) as stains. Histomorphometric studies were performed using Olympus light microscope (x100) fitted with Casio digital camera and Motic plus China, 2000 software.

## 3.8. STATISTICAL ANALYSIS

Data were presented as Mean ± SEM for 8 animals. Statistical significance between groups was evaluated using analysis of variance (ANOVA) and Student's t-test for paired data with the aid of Graphpad Prism 5. In all data analysis, $P = 0.05$ was considered significant.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Effect of risperidone on water immersion restraint stress-induced gastric ulceration (Ulcer Scores)

From Table 3, there wns a dose dependent decrease in the ulcer scores of the risperidone pretreated groups; 0.1mg/kg (3.50± 0.19), 0.1mg/kg (1.88± 0.30) and 0,5mg/kg (1.23± 0.18) compared to the control (5.56± 0.32). The percentage inhibition of ulcer was also dose-dependent: 0.1mg/kg (37.08%), 0.3mg/kg (66.30%) and 0.5mg/kg (79.78%). The degree of inhibition of ulceration is an index of ulcer protection. This increase was found to be well correlated with increasing doses of risperidone. This is indicative of increase in degree of protection with increase in dose of risperidone.

### 4.2 Effect of risperidone on water immersion restraint stressinduced gastric ulceration

Plate 5A (control) showed extended haemorrhagic ulcers greater than 3 mm in length in most portions of the stomach compared to the risperidone pretreated animal, A (0.1 mg/kg), B (0.3 mg/kg) and C (0.5 mg/kg). Fewer numbers of extended haemorrhagic ulcers were recorded in Plate 5B. These were for animal treated with 0.1 mg/kg risperidone, while in Plate 5C, there are remarkably very low ulcers spots with only one extended haemorrhagic ulcer. Plate 5D animals treated with 0.5 mg/kg had little ulcer. The decreases in ulceration in the risperidone-pretreated rat groups are significant compared to the control ($p \leq 0.5$).

**Table 3: Effect of risperidone on water immersion restraint stress (WIRS)- induced gastric ulceration**

| Treatment | Ulcer score[a] | Inhibition of ulceration (%)[b] |
|---|---|---|
| Normal saline (Control) | 6.06 ± 0.32 | - |
| Risperidone (0.1mg/kg) | 4.00 ± 0.30* | 33.99 |
| Risperidone ( 0.3mg/kg) | 2.25 ± 0.21* | 62.87 |
| Risperidone (0.5mg/kg) | 1.75 ± 0.21* | 71.12 |

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

Inhibition of ulceration (%)[b] = $\frac{\text{Control} - \text{Treatment} \times 100}{\text{Control}}$

([b]Percentage inhibition as described by Raji *et al.* 2000).

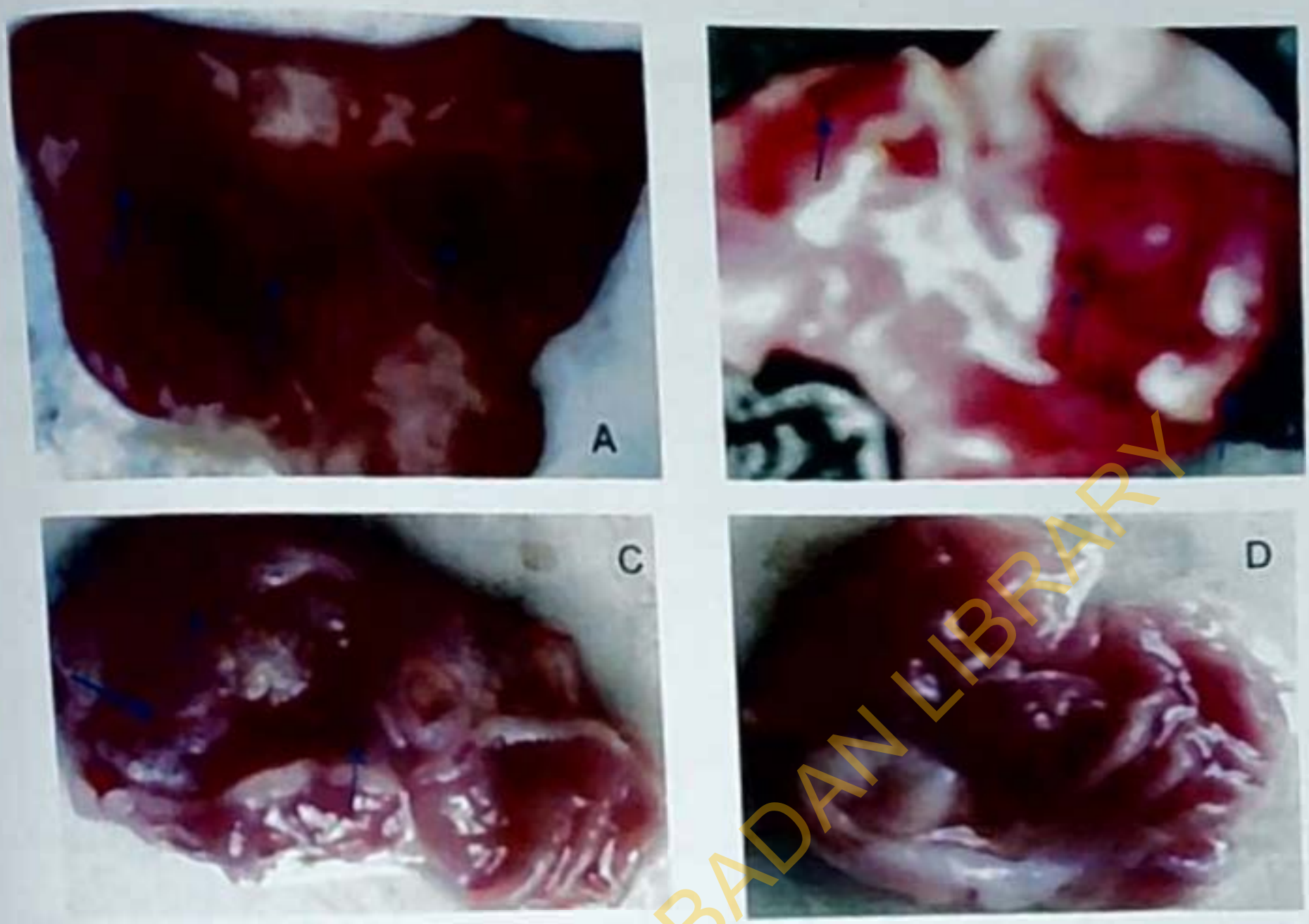


**Plate 5. Macroscopic changes on the effect of risperidone-treated rats on WIRS-induced gastric ulceration (x100)**

A Control group (WIRS alone) - Evidence of severe Ulcer seen
B (0.1mg kg Risp + WIRS) - ulcer seen are less severe
C (0.3 mg kg Risp + WIRS) – two or more pin point ulcer present
D (0.5 mg kg Risp + WIRS) – Pin point ulcer

### 3 Effect of risperidone on water immersion restraint stress-induced gastric ulceration

There was dose-dependent decrease in ulcer scores for animals in the 0.1mg/kg, 0.3mg/kg and 0.5mg/kg. Figure 17 shows decrease in gastric ulceration as the dose of the risperidone increased and these are graded and dose dependent decrease in ulcer scores for the 0.1mg (4.0 ± 0.30), 0.3mg/kg (2.25 ± 0.21) and 0.5mg/kg (1.75 ± 0.21) animals groups. The decreases in ulcer in the experimental groups were significantly reduced compare to control (6.06 ± 0.32) ($p \leq 0.5$).

### 4.4 Ulcer area in water immersion restraint stress-induced gastric ulceration in risperidone treated rats

The ulcer area $(\mu m)^2$ in the 0.1mg/kg (1,883,950), 0.3mg/kg (369,991) and 0.5mg/kg (85,150) risperidone pretreated animals showed a dose-dependent decrease compared to the control (3,653,500) – (Table 4). This decrease in ulcer area was significantly different from that in control animals with each of the doses ($p \leq 0.05$). There were also significant dose-dependent decreases in the % ulcer area and perimeter (μm) in the risperidone group compared to the control.

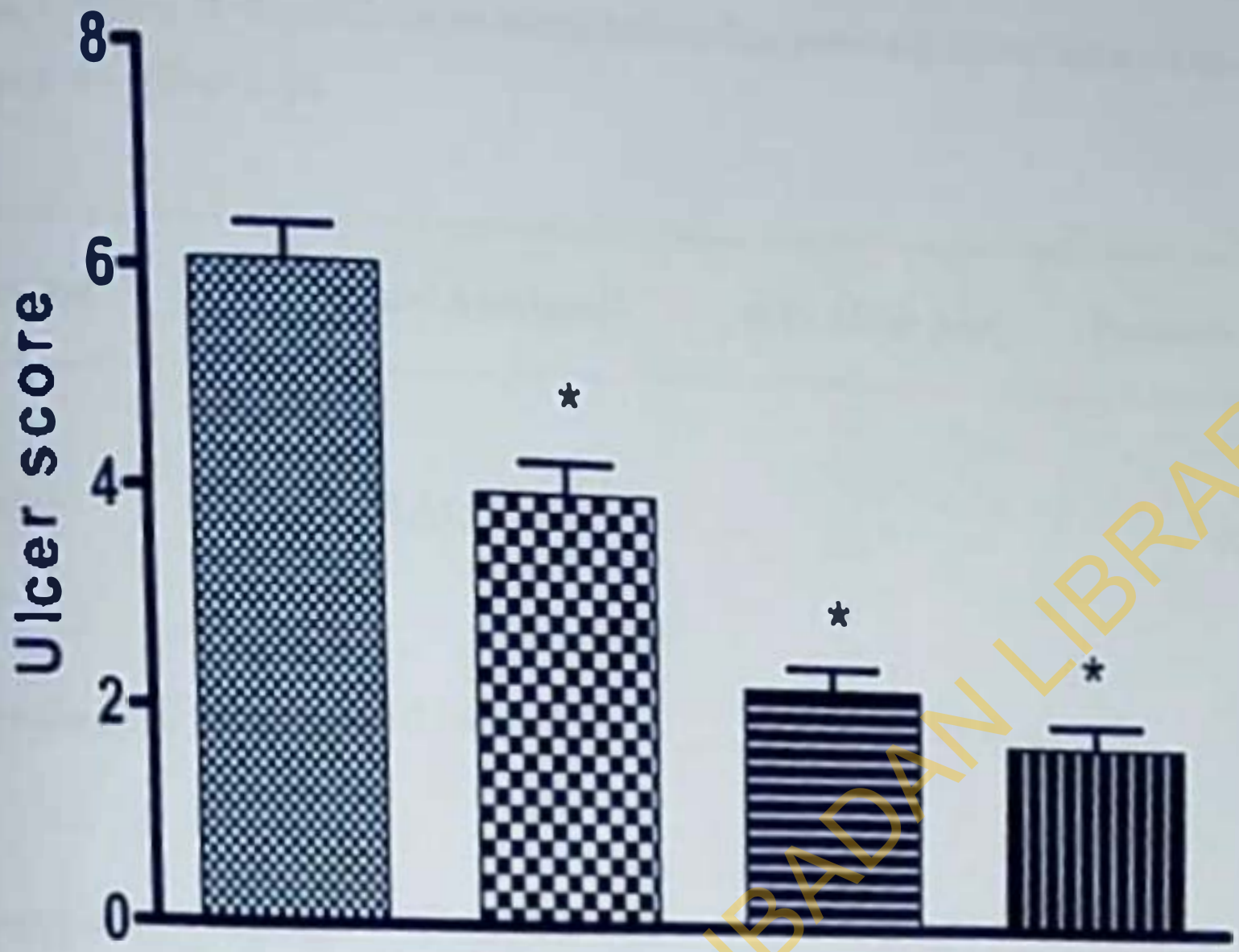


Figure 17 Effect of risperidone on WIRS-induced gastric ulcer in rats

Values are Mean ± SEM for 8 animals per group. P<0.05 significantly lower compared with control

**Table 4: Effect of risperidone on water immersion restraint stress induced gastric ulceration – Ulcer area**

| Treatment | Ulcer Area $(\mu m)^2$ | % of Ulcer Area | Perimeter ($\mu m$) |
|---|---|---|---|
| Control | 3,653,500.0 | - | 10,846.1 |
| Risperidone (0.1mg/kg) | 1,883,950.0 | 51.57 * | 6,706.6 |
| Risperidone (0.3mg/kg) | 369,991.0 | 10.13* | 5,987.0 |
| Risperidone (0.5mg/kg) | 85,150.0 | 2.33* | 2,447.0 |

* The percentage of ulcer area (an index of ulceration) indicated above showed dose-dependent relationship with the doses of risperidone. The dose-dependent relation is significant compared to the control

### .5 Effect of risperidone on indomethacin-induced gastric ulceration (Ulcer scores)

There were dose –dependent decrease in the ulcer scores of the risperidone treated group; 0.1mg/kg (4.94± 0.26), 0.1mg/kg (2.00± 0.19) and 0,5mg/kg (1.31± 0.19) compared to the control (6.44± 0.36) - (Table 5). The percentage inhibition increase with increase in doses of risperidone: 0.1mg/kg (23.30%), 0.3mg/kg (68.93%) and 0.5mg/kg (79.61%). This is an indicative of increase in degree of gastroprotection.

### 4.6 Effect of risperidone on Indomethacin-induced gastric ulceration

Plate 6 shows ulcer lesions (shown by the arrows) of the control (A) compared to the risperidone treated animal. A (0.1mg/kg), B (0.3mg/kg) and C (0.5mg/kg). Plates 6C and 6D showed fairly intact, however with dotted patched of ulcers. Plate 6D is almost free of ulcers. The ulceration decreases as the dose of risperidone increased compared to the control ($p \leq 0.5$).

Table 5: Effect of risperidone on indomethacin-induced gastric ulceration

| Treatment | Ulcer score[a] | Inhibition of ulceration (%)[b] |
|---|---|---|
| Distilled water (Control) | 6.44 ± 0.36 | - |
| Risperidone (0.1mg/kg) | 4.94 ± 0.26* | 23.30 |
| Risperidone (0.3mg/kg) | 2.00 ± 0.19* | 68.93 |
| Risperidone (0.5mg/kg) | 1.31 ± 0.19* | 79.61 |

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

$$\text{Inhibition of ulceration (\%)}^{b} = \frac{\text{Control} - \text{Treatment} \times 100}{\text{Control}}$$

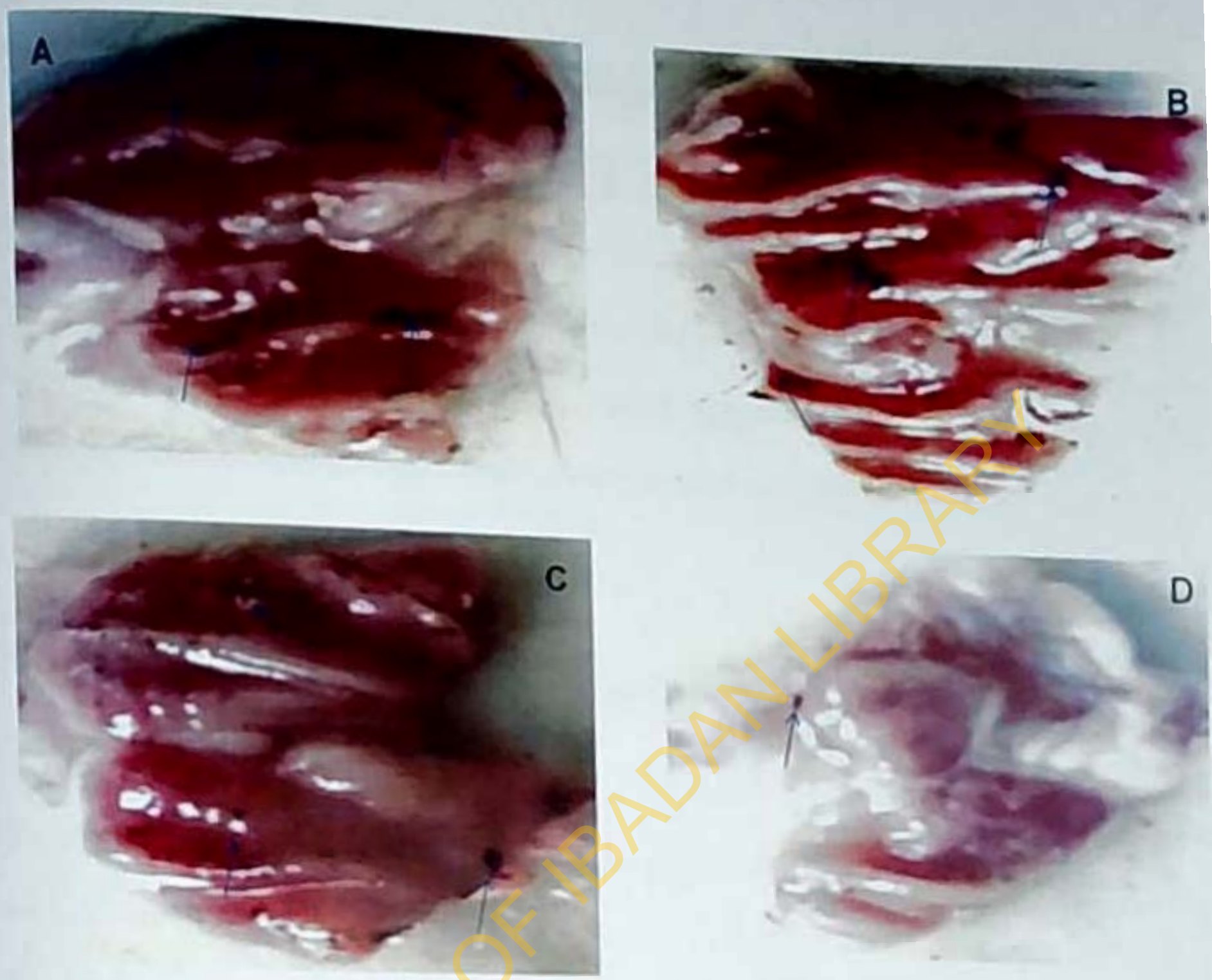


**Plate 6. Effect of risperidone on indomethacin-induced gastric ulceration**

A Control group - indomethacin alone - Very severe ulcer of more than 3mm

B Risperidone (0.1 mg/kg) + indomethacin - Less severe ulcer of more than 3mm

C Risperidone (0.3 mg/kg) + indomethacin - Mild ulcer

D Risperidone (0.5 mg/kg) + Indomethacin - Lesser or no ulcer

### 7 Effect of risperidone on indomethacin-induced gastric ulceration

The figure 18 shows the graph of ulcer scores against dose of risperidone. The risperidone treated animals ulcer scores are 0.1mg/kg (4.93 ± 0.26), 0.3mg/kg (2.00 ± 0.19) and 0.5mg/kg (1.31±0.19) compared to the control (6.438 ± 0.36). It shows a graded and dose-dependent decrease in the mean ulcer scores with increases in dose of risperidone. These decreases in all the risperidone treated animals are significantly different from the control ($p \leq 0.05$).

### 4.8 Effect of risperidone on indomethacin-induced gastric ulceration (ulcer area)

The ulcer area $(\mu m)^2$ and percentage of ulcer area (%) in the 0.1mg/kg (636,592.6 and 23.91), 0.3mg/kg (489,350.0 and 18.18) and 0.5mg/kg (242,750.0 and 9.12) risperidone pretreated animals showed a dose-dependent decrease compared to the control (2,662,067.0). This decrease is significantly different from the control ($p \leq 0.05$). There was also significant dose-dependent decreases in the perimeter (μm) in the risperidone pretreated group compared to the control (Table 6).

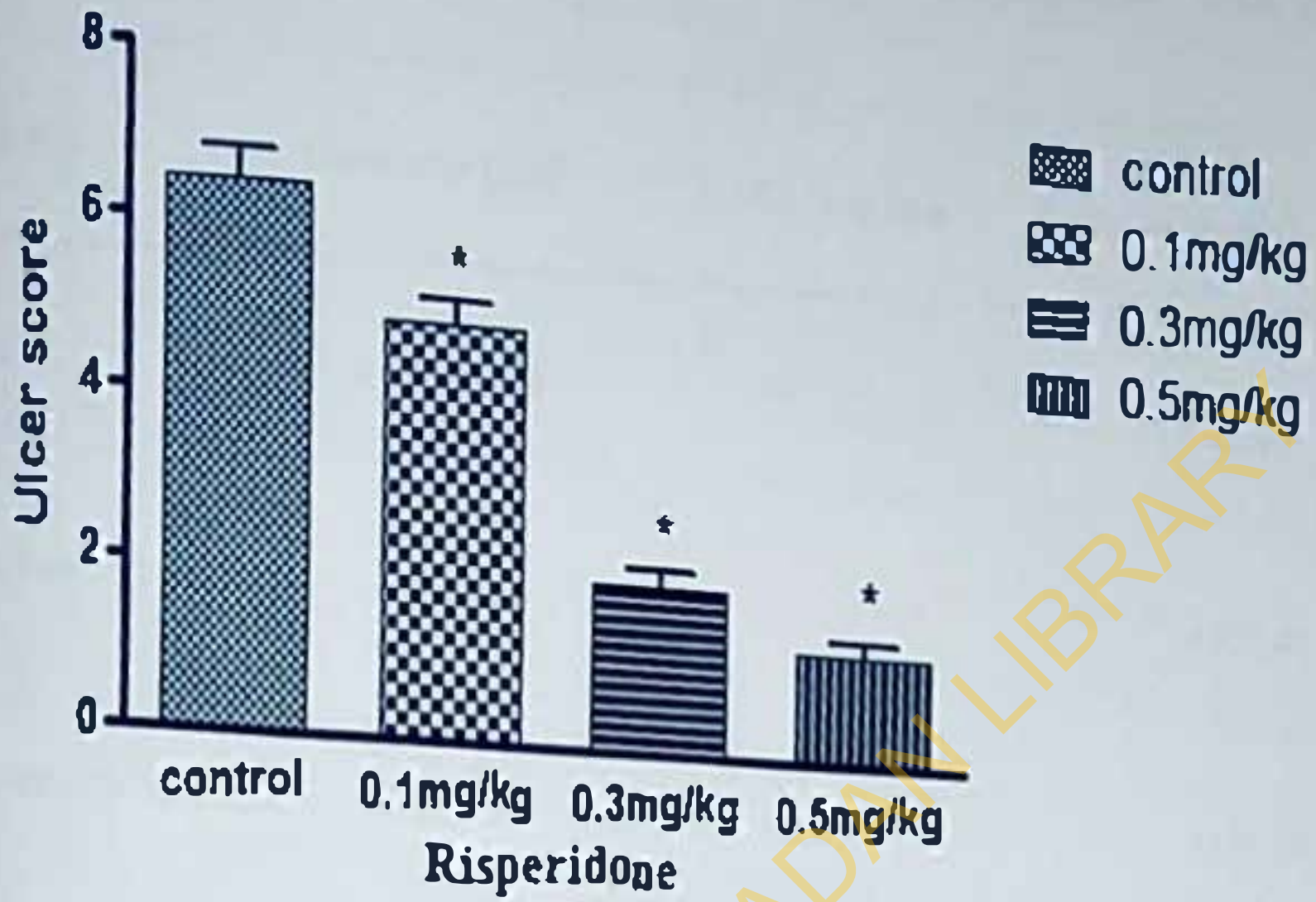


Figure 18 Risperidone reduces ulcer scores in indomethacin treated rats

**Table 6: Effect of risperidone on indomethacin- induced gastric ulceration – ulcer area**

| Treatment | Ulcer area $(\mu m)^2$ | % of Ulcer area | Perimeter (µm) |
|---|---|---|---|
| Control | 2,662,067.0 | - | 11,320.0 |
| Risperidone (0.1mg/kg) | 636,592.6 | 23.91• | 4722.4• |
| Risperidone (0.3mg/kg) | 489,350.0 | 18.38• | 4372.6• |
| Risperidone (0.5mg/kg) | 242,750.0 | 9.12• | 3987.8• |

The table 6 shows the area of ulceration in the indomethacin-induced gastric ulceration. There is a decrease with an increase in dose of risperidone. The dose-dependent relation is significant• compared to the control.

### 9 Effect of risperidone on starvation-induced gastric ulceration (ulcer score)

From table 7, there was a dose -dependent decrease in the ulcer scores of the risperidone pretreated group 0.1mg/kg (3.50± 0.19), 0.1mg/kg (1.88± 0.30) and 0.5mg/kg (1.13± 0.18) compared to the control (5.56± 0.32). The percentage inhibition increases with increase in dosage of risperidone: 0.1mg/kg (37.05%), 0.3mg/kg (66.19%) and 0.5mg/kg (79.68%). This is indicative of increase in degree of protection with increase in dose of risperidone.

### 4.10 Effect of risperidone on starvation-induced gastric ulceration

Plate 7 shows ulcer lesions (shown by the arrows) of the control (A) compared to the risperidone pretreated animal, A (0.1mg/kg), B (0.3mg/kg) and C (0.5mg/kg). There are decreases in ulceration as the dose of risperidone increased compared to the control ($p \leq 0.5$).

**Table 7: Effect of risperidone on starvation (6d) - induced ulceration**

| Treatment | Ulcer score[a] | Inhibition of ulceration (%)[b] |
|---|---|---|
| Distilled water (Control) | 5.56 ± 0.32 | - |
| Risperidone (0.1mg/kg) | 3.50 ± 0.19* | 37.05 |
| Risperidone (0.3mg/kg) | 1.88 ± 0.30* | 66.19 |
| Risperidone (0.5mg/kg) | 1.13 ± 0.18* | 79.68 |

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

[b]Inhibition of Ulceration (%) = $\frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$

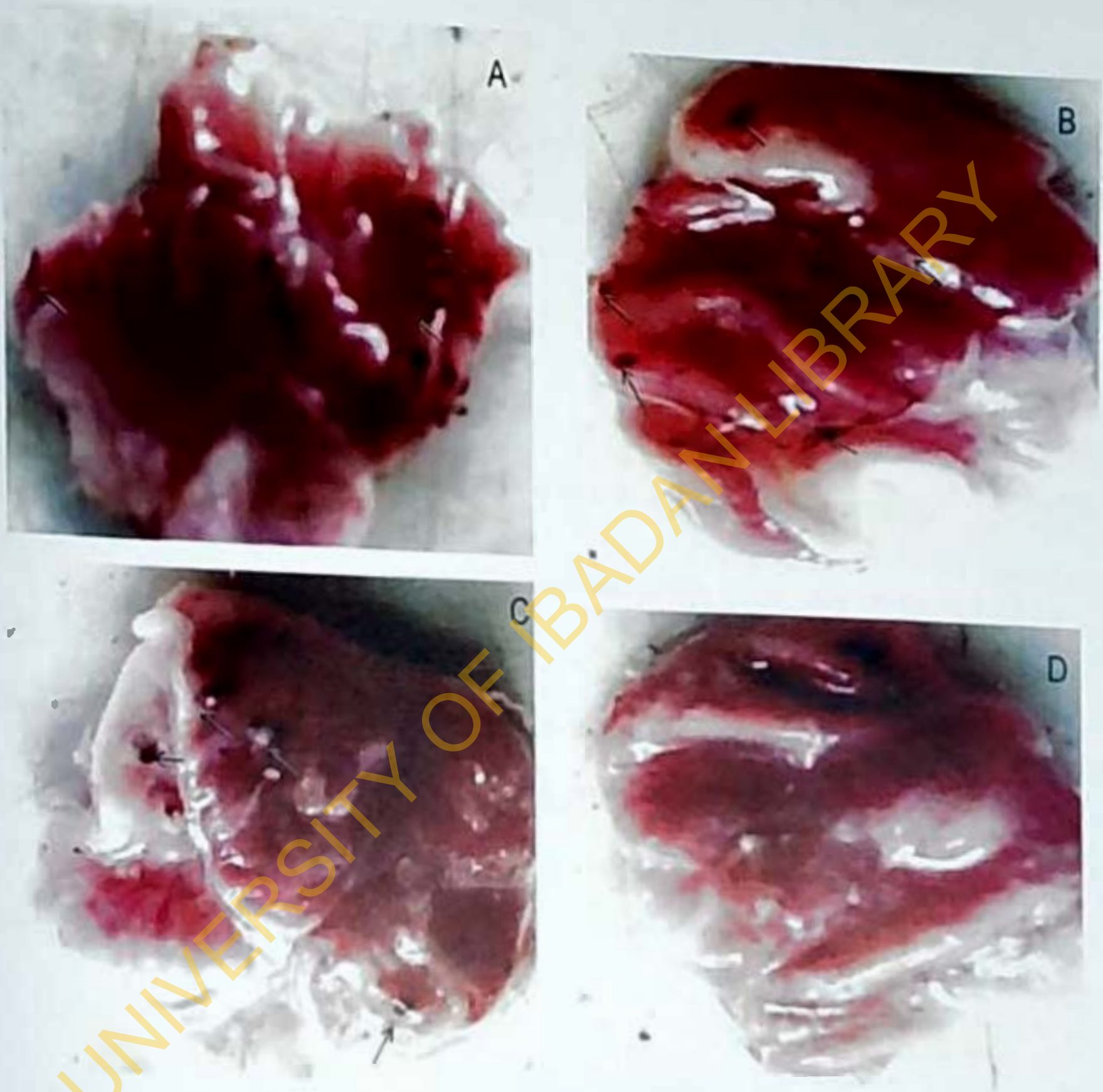


**Plate 7: Effect of risperidone on starvation induced ulceration**

A Control group (starvation alone) - severe ulcer

B Risperidone (0.1mg/kg) + starvation - less severe ulcer

C Risperidone (0.3 mg/kg) + starvation - mild ulcer

D Risperidone (0.5 mg/kg) + starvation - less number of ulcer

### 4.11 Effect of risperidone on starvation-induced gastric ulceration (ulcer scores)

The figure 19 shows the graph of ulcer scores against dose of risperidone in starvation-induced gastric ulceration. It shows a dose-dependent decrease in the ulcer score with increase in dose of risperidone. These decreases in all the risperidone treated animals are significant compared to the control ($p \leq 0.05$).

### 4.12 Effect of risperidone on starvation-induced gastric ulceration (ulcer area)

As shown in Table 8, the ulcer area $(\mu m)^2$ and percentage ulcer area in the 0.1mg/kg (500,200.0 and 24.09), 0.3mg/kg (228,500.0 and 11.0) and 0.5mg/kg (116,600.0 and 5.62) risperidone pretreated animals showed a dose-dependent decrease compared to the control (2,076,500.0). This decrease is significantly different compared to the control ($p \leq 0.05$). There were also significant dose-dependent decreases in the perimeter (μm) in the risperidone pretreated group compared to the control.


Figure 19 Ulcer score against risperidone in starvation-induced gastric ulcer

**Table 8: Effect of risperidone on starvation- induced gastric ulceration (Ulcer area)**

| Treatment | Ulcer area $(\mu m)^2$ | % of Ulcer area | Perimeter (μm) |
|---|---|---|---|
| Control | 2,076,500.0 | - | 8,430.4 |
| Risperidone (0.1mg/kg) | 500,200.0 | 24.09* | 3457.7* |
| Risperidone (0.3mg/kg) | 228,500.0 | 11.00* | 2888.0* |
| Risperidone (0.5mg/kg) | 116,600.0 | 5.62* | 2188.3* |

Table 8 shows the area of ulceration in the starvation-induced gastric ulceration. There is a decrease with an increase in dose of risperidone. The dose-dependent relation is significant* compared to the control ($p \leq 0.05$).

## 4.13 Effect of risperidone on histamine-induced GAS

Results obtained from this study (Figure 20) showed that risperidone decreased gastric acid secretion in experimental animals in a dose dependent manner compared with the controls. There were significant reductions in gastric acid secretion after histamine administration compared with control ($p \leq 0.05$). Between 0-40 minutes, the basal secretion was fairly normal in all the groups. After 40 minutes, histamine was administered intravenously through the femoral vein and 10 minutes later, there was a sharp increase in gastric acid secretion in the control rats, while the risperidone-treated groups showed a fall in gastric acid secretion that were all significantly different ($p \leq 0.05$) compared to those of the control groups. Analysis of the 50th – 70th minute's interval also showed a significant decrease in the risperidone treated rats compared to the control rats group.

## 4.14 Effect of risperidone on pentagastrin induced gastric acid secretion

There were significant reductions in gastric acid secretion after pentagastrin (25 µg/kg, i.p.) administration when compared to the control ($p \leq 0.05$). Between 0-40 minutes, the basal secretion was rising fairly constant in their secretions in all the groups (figure 21). After the 40th minutes, pentagastrin a known secretagogue was administered intraperitoneally. After 10 minutes, there was a sharp increase in gastric acid secretion in the control rats, while the risperidone-treated groups showed a fall in gastric acid secretion that were all significantly different ($p \leq 0.05$) compared to those of the control groups. Analysis of the 50th – 80th minute's interval also showed a significant decrease in the pretreated rats compared to the control rats group.

minute's interval also showed a significant decrease in the pretreated rats compared to the control rats group.

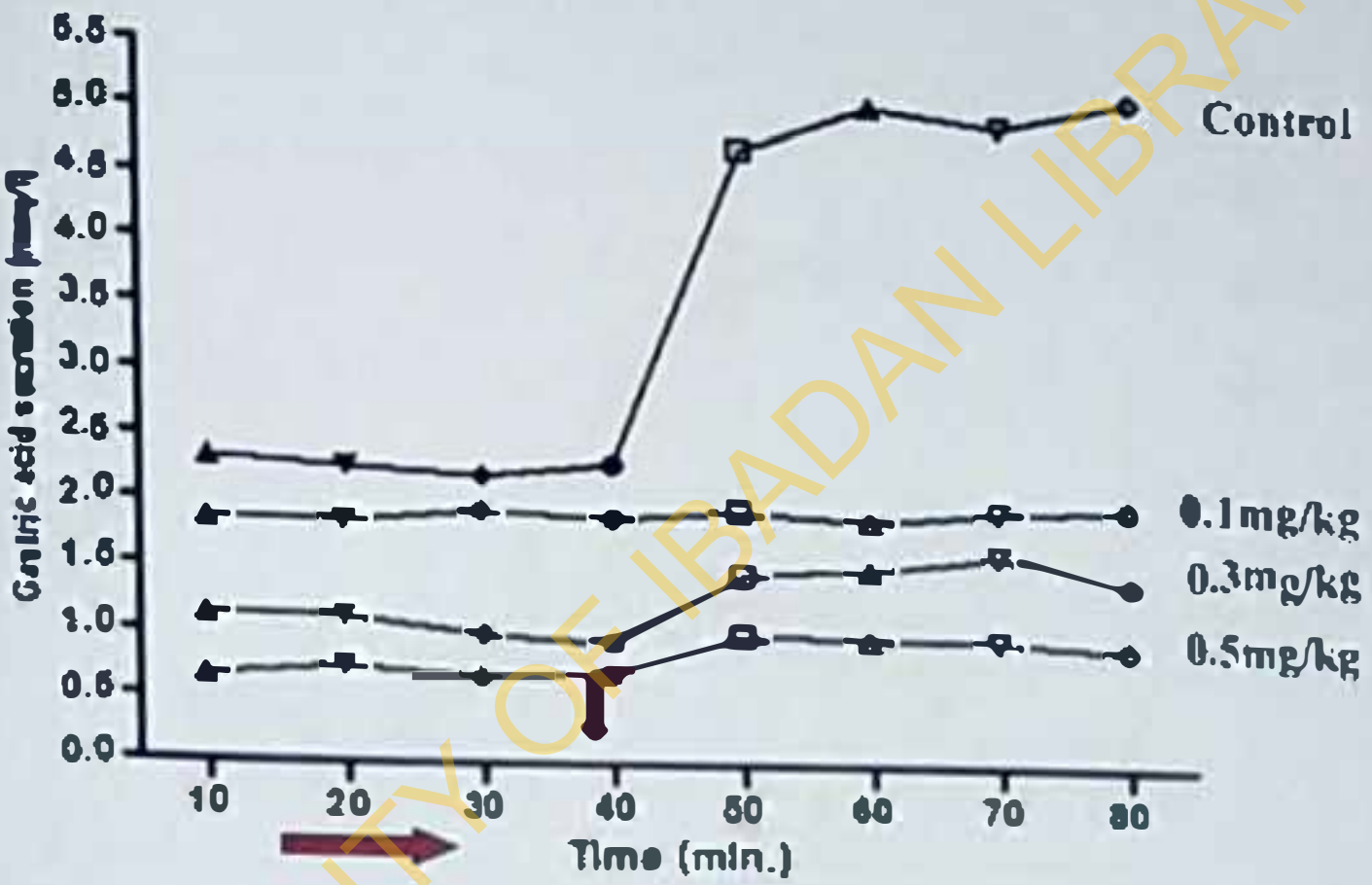


**Figure 16 Effect of risperidone on gastric acid secretion induced by histamine in rats**

= Before arrow shows basal secretion.

= Arrow indicates point of injection of histamine administration, i.v.

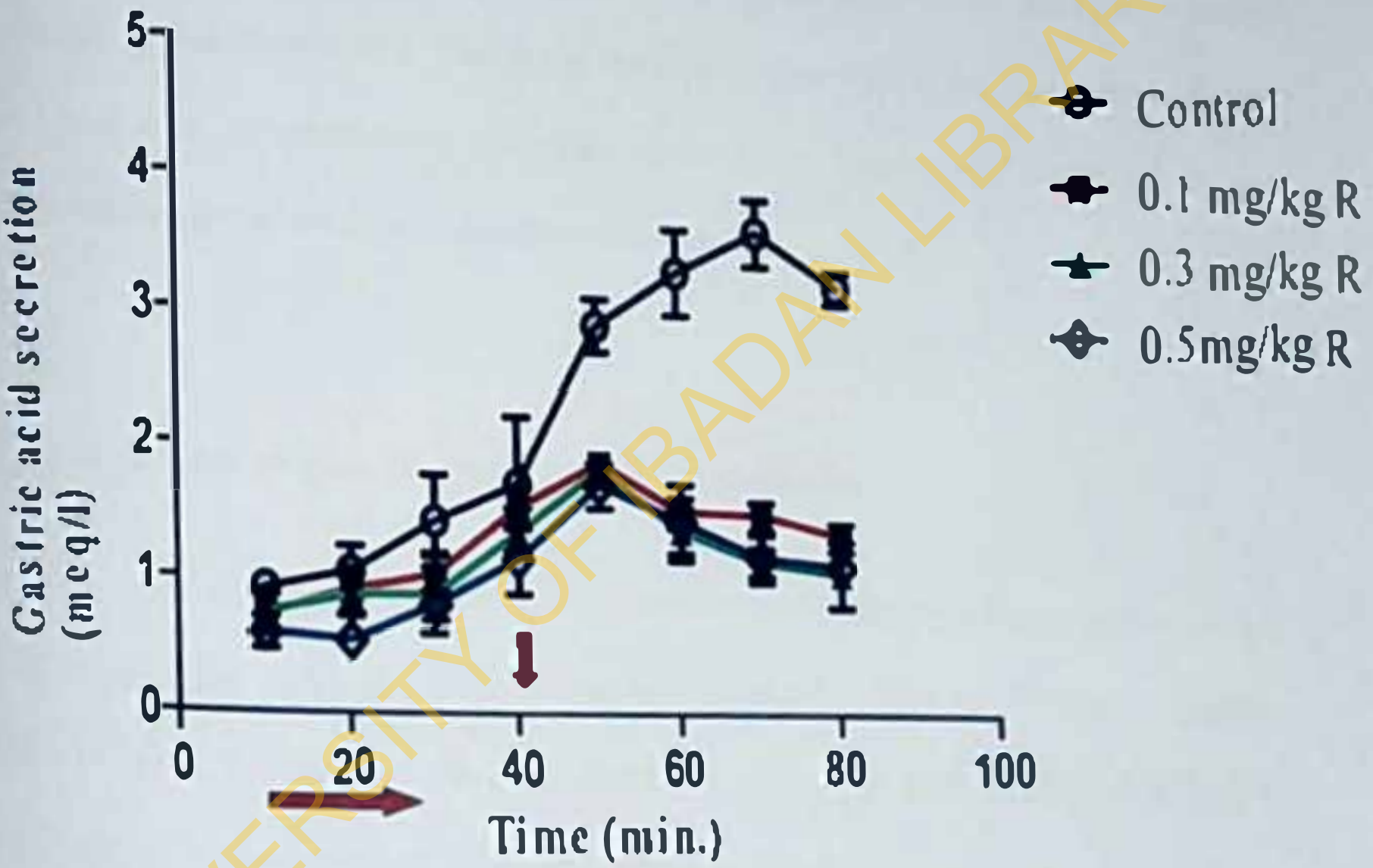


**Figure 17 Effect of risperidone on pentagastrin induced gastric acid secretion**

= Before arrow shows basal secretion.

= Arrow indicates point of injection pentagastrin administration, *i.p.*

## 4.15 Effect of risperidone on carbachol induced gastric acid secretion

There were no significant reductions in gastric acid secretion after carbahcol (4 µg/kg, *i.p.*) administration when compared to the control ($p \geq 0.05$). Between 0-40 minutes, the basal secretion was similar in all the groups (figure 22). After the 40th minutes, carbacol was administered intraperitoneally (*i.p.*). Within 10 minutes there was a sharp increase in gastric acid secretion in the control rats. The same trend was observed in the risperidone treated groups. There was no significant difference ($p \geq 0.05$) in gastric acid secretion in the risperidone-treated groups compared to those of the control.

## 4.16 Effect of risperidone on gastric mucus secretion

In Figure 23, there was significant increase in gastric mucus secretion (mg/g tissue) in the 0.1mg/kg risperidone (1.06 ± 0.11), 0.3mg/kg risperidone (1.31±0.12) and 0.5mg/kg risperidone (1.39±0.20) pretreated rats compared to the control rats 0.60± 0.03 ($p \leq 0.05$). This increase in gastric mucus secretion is dose-dependent.

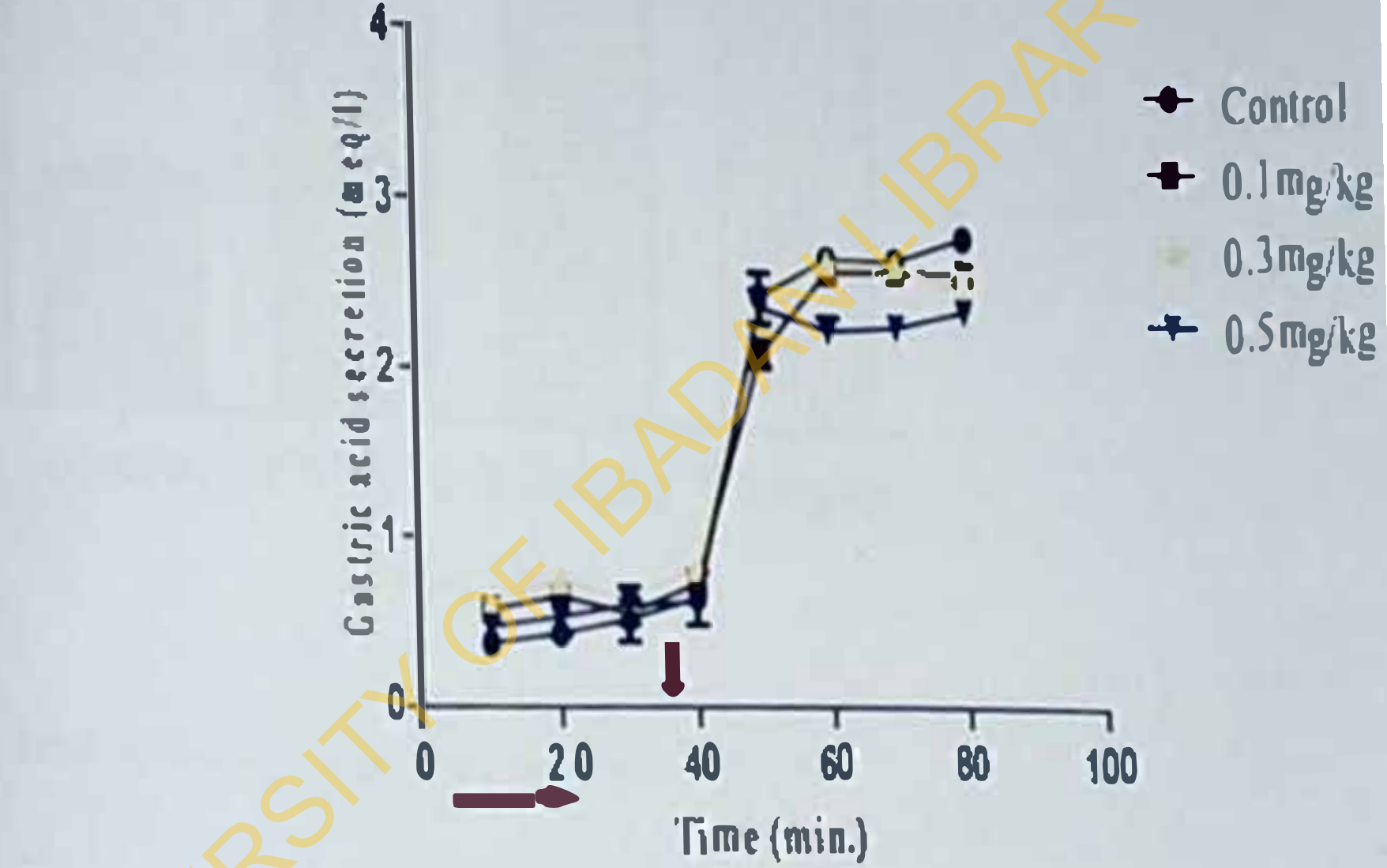


Figure 18 Effect of risperidone on carbachol induced gastric acid secretion

= Before arrow shows basal secretion.

= Arrow indicates point of injection of carbacol administration, i.p

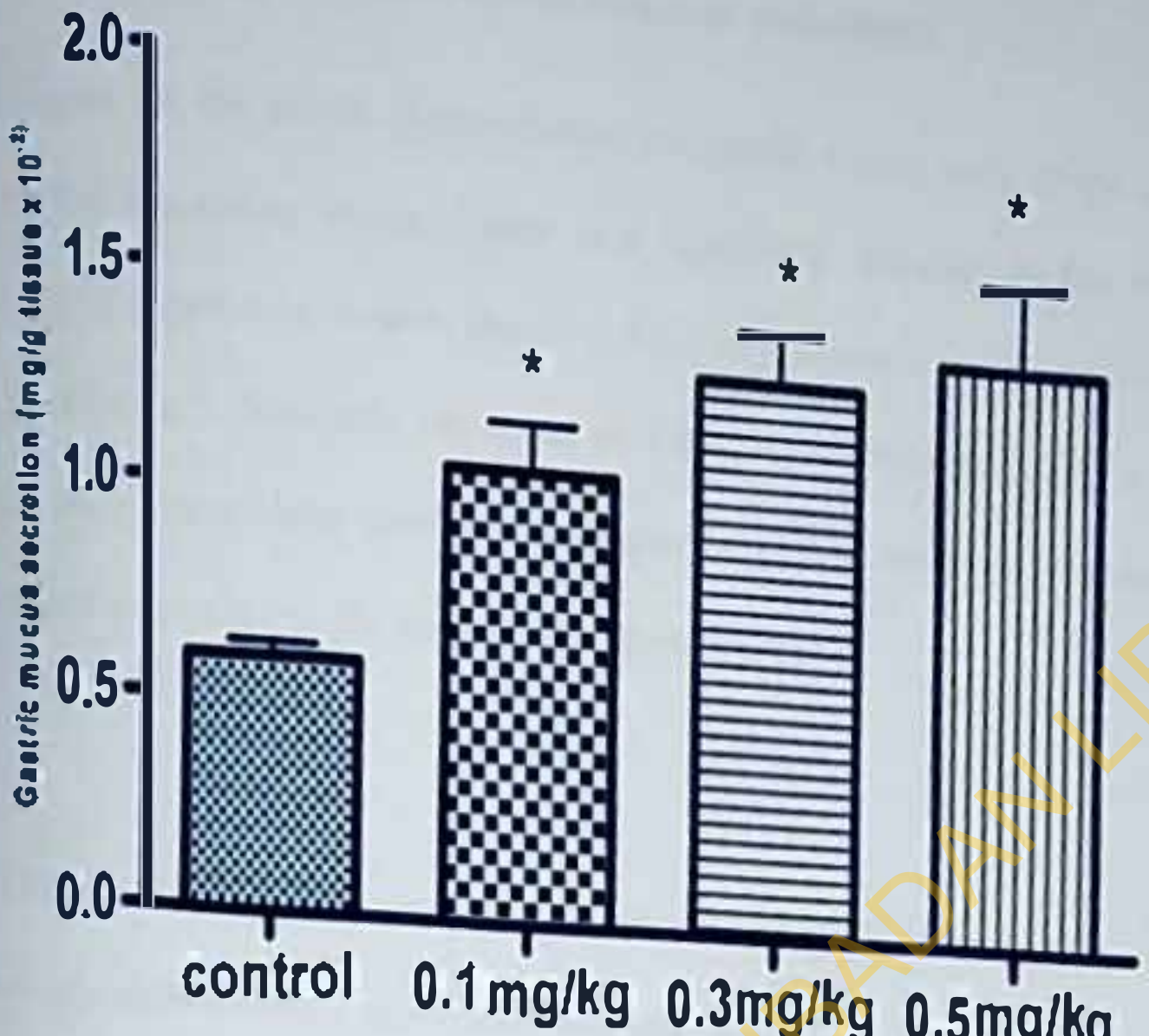


Figure 23: Effect of risperidone on gastric mucus secretion

## 4.17 Effect of risperidone on gastric mucus cells count

In Figure 24, the effect of risperidone on gastric mucus cells count showed graded increase with the increasing doses. There is a significant increase in the mucus cells count with 0.1mg/kg risperidone treated rats (121.2± 5.04 cells/cm$^2$) compared to the control rats (103.3± 4.18 cells/cm$^2$). Similarly the doses of 0.3mg/kg (128.6± 2.46) and 0.5 mg/kg (129.3 ± 3.73 cells/cm$^2$) risperidone produced increases in gastric mucus cells count that are significantly different compared to the control rats group ($p<0.05$)

## 4.18 Effect of risperidone on indomethacin, SC-560 and celecoxib combination, SC-560 and celecoxib induced gastric ulceration

The figure 25 shows the ulcer scores against indomethacin (40mg/kg), SC-560 and celecoxib combination, risperidone and indomethacin, risperidone + SC-560 + celecoxib combination, risperidone + SC-560 combination and risperidone + celecoxib combination -induced gastric ulceration. It shows a decrease in the ulcer score in the combined doses of risperidone and SC-560 + celecoxib. These decreases showed significant difference compared to those of indomethacin, SC-560 and celecoxib.

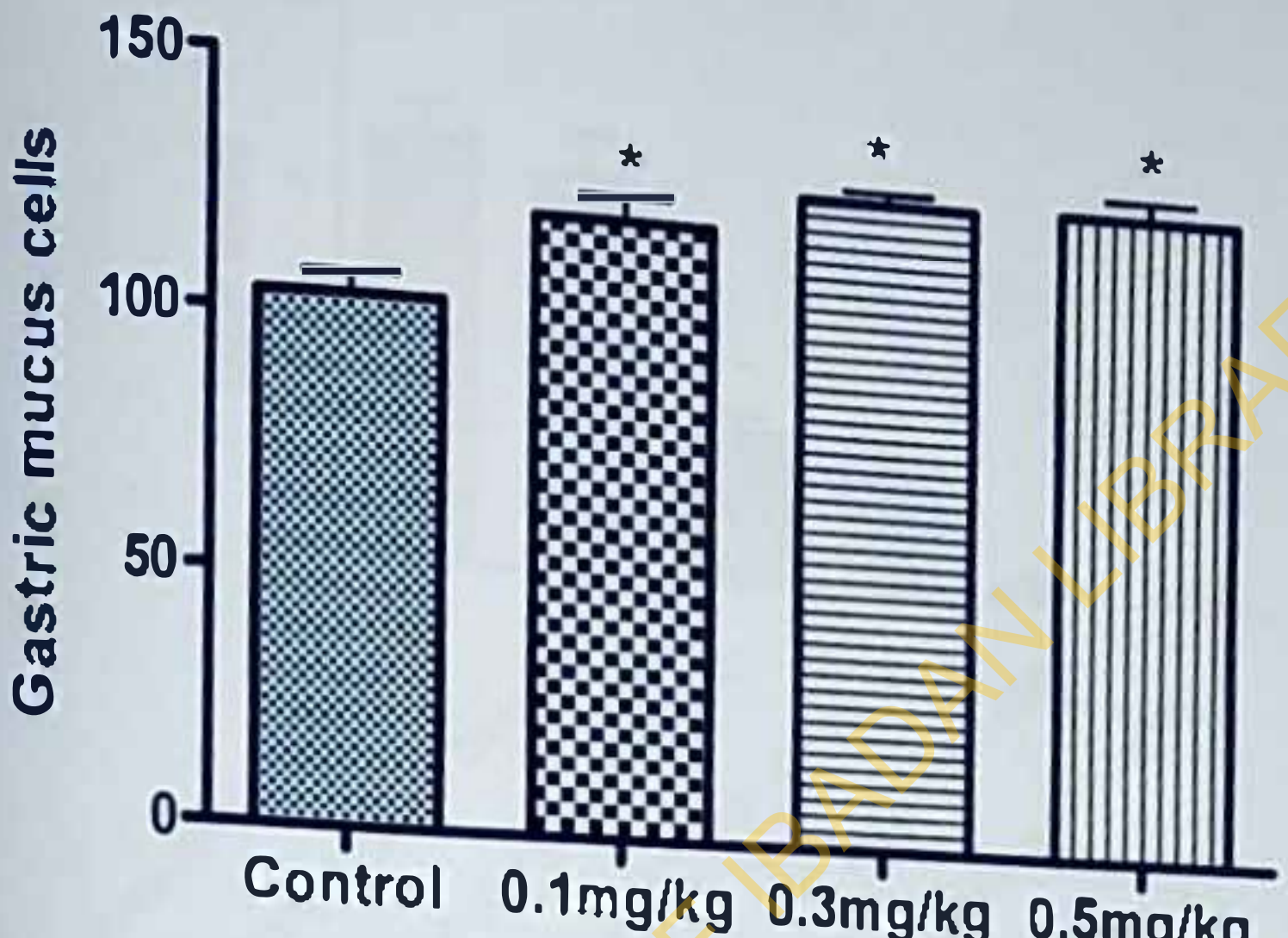


Figure 24: Effect of risperidone on gastric mucus cells count

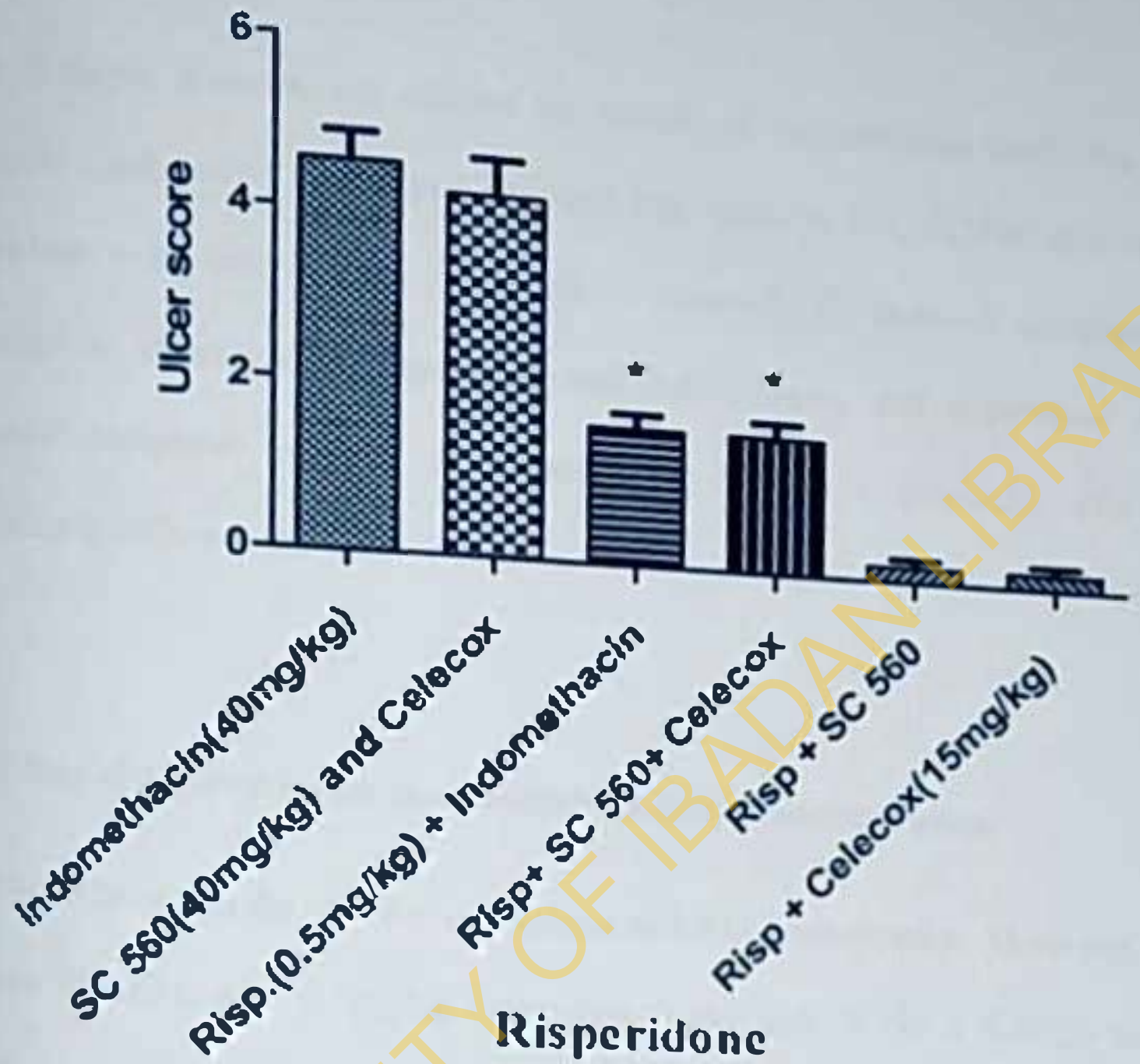


Figure 25. Effect of risperidone on indomethacin, SC-560 and celecoxib combination, SC 560 and celecoxib- induced gastric ulcer

## 4.19 Effect of risperidone on indomethacin, cyclooxygenase 1(SC -560) and 2 (celecoxib) inhibitors induced gastric ulceration

Plate 8 shows ulcer lesions (shown by arrows) of indomethacin only (A), SC-560 and celecoxib combination (B), risperidone and indomethacin (C), SC-560 and celecoxib (D), risperidone + SC-560 (E) and risperidone + celecoxib (F) induced ulceration. There are decreases in ulceration in risperidone and indomethacin, and risperidone + SC-560 + celecoxib compared to those of indomethacin, SC-560 + celecoxib. This decrease is significantly different ($p \leq 0.5$).

## 4.20 Effect of risperidone on malonialdehyde (MDA) concentration

The Figure 26 shows the effect of risperidone on MDA concentration. There was a significant decrease in MDA with 0.1mg/kg risperidone treated rats (0.194 ± 0.0072) as against the control rats (0.257 ± 0.0087) ($p \leq 0.05$). The concentration of MDA concentration in the 0.3mg/kg (0.183 ± 0.0073) and the 0.5mg/kg (0.106 ± 0.0049) risperidone treated rats showed significant decrease compared to the control ($p \leq 0.05$). This decrease exhibits dose dependence.

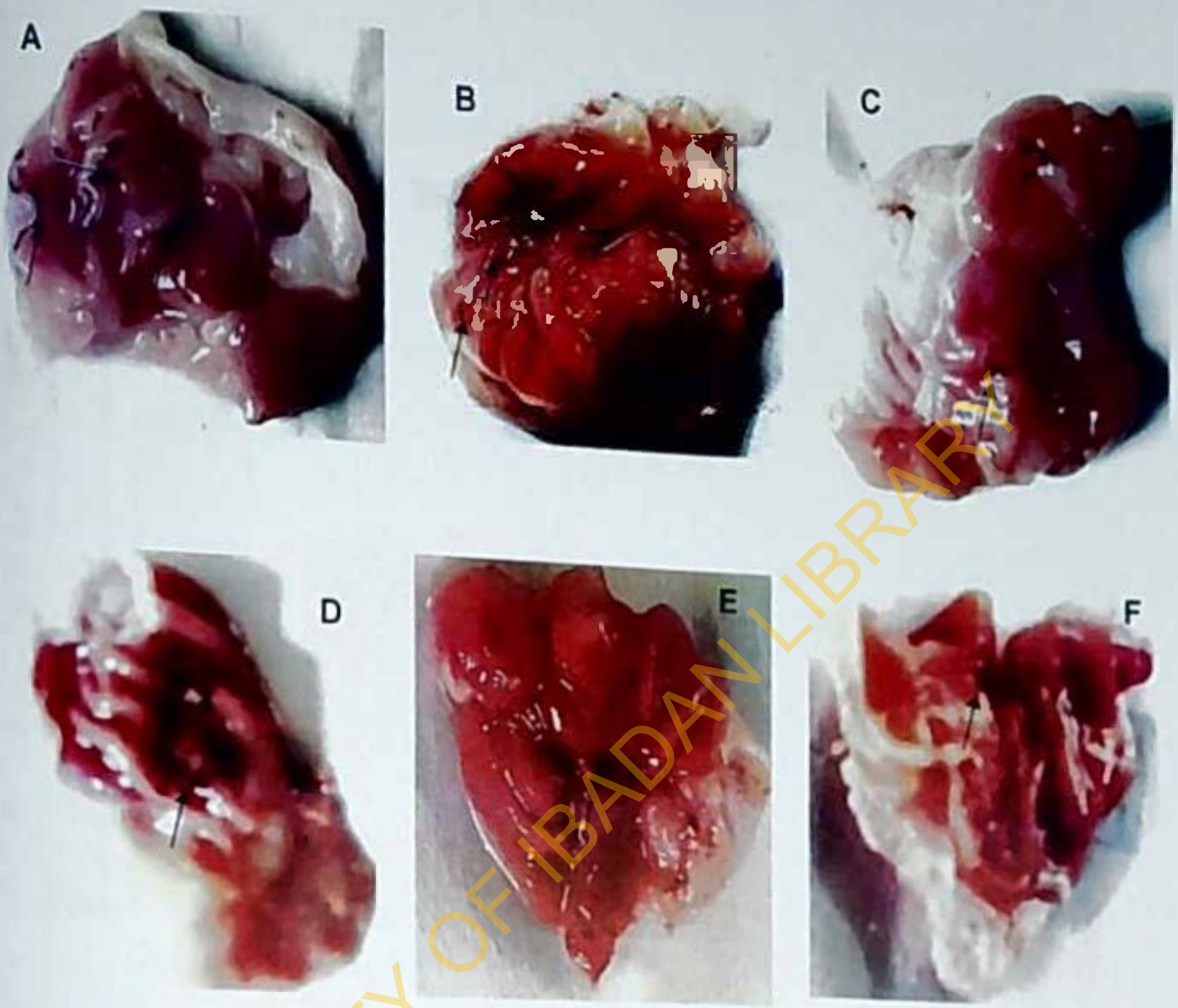


**Plate 8: Effect of risperidone on indomethacin, cyclooxygenase 1(SC -560) and 2 (celecoxib) inhibitors induced gastric ulceration**

A Indomethacin only

B SC-560 and celecoxib combination

C Risperidone and indomethacin

D Risperidone + SC-560 + celecoxib

E Risperidone + SC-560

F Risperidone + celecoxib

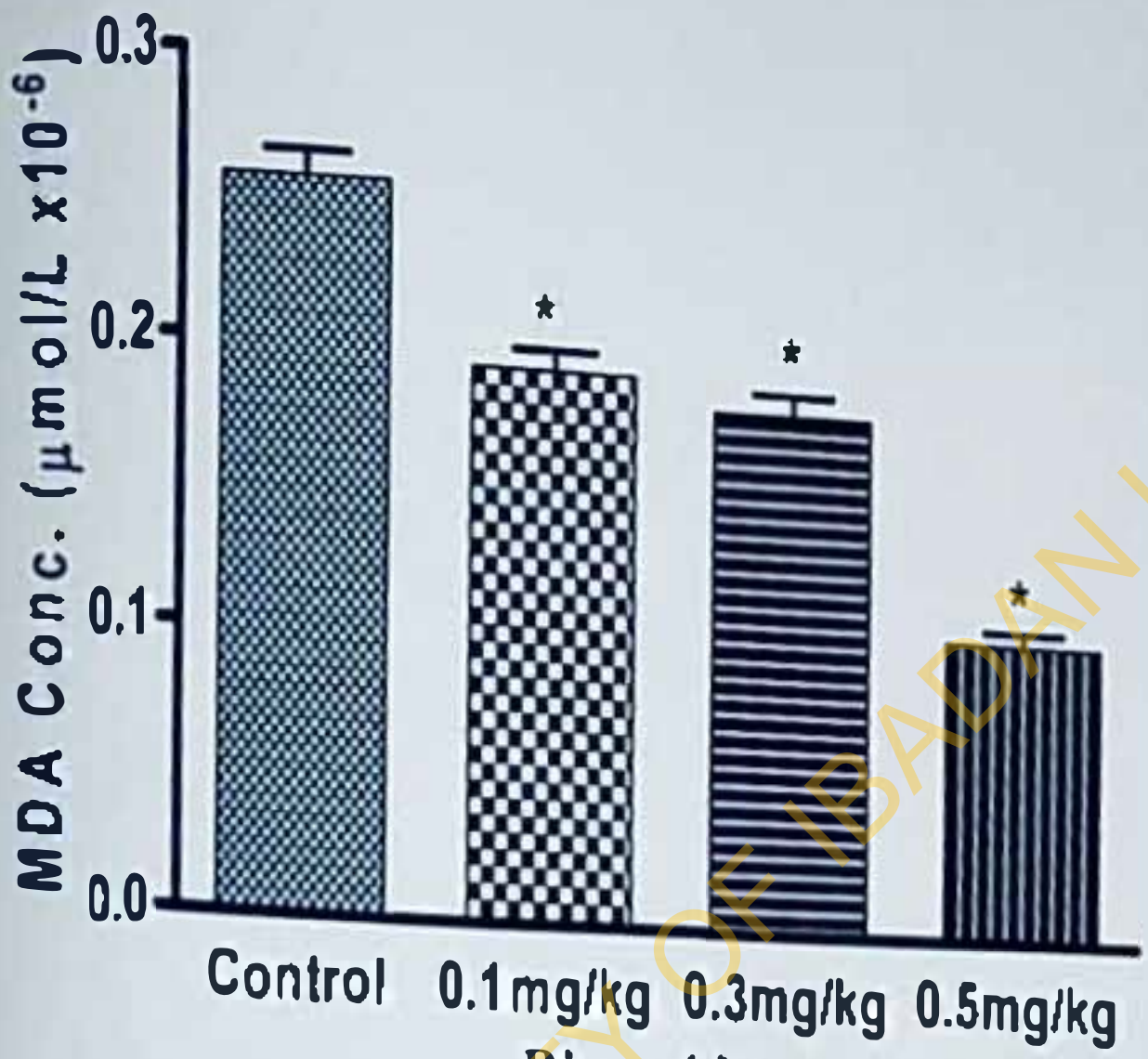


Figure 26 Effect of risperidone on malonialdehyde (MDA) concentration

**4.21. Effect of risperidone on the histological changes of the gastric mucosal.**

Plate 9 showed the effect of risperidone on all the different ulcer models. There was evidence of reduction in gastric ulceration in all the risperidone pretreated rats groups compared to the controls.

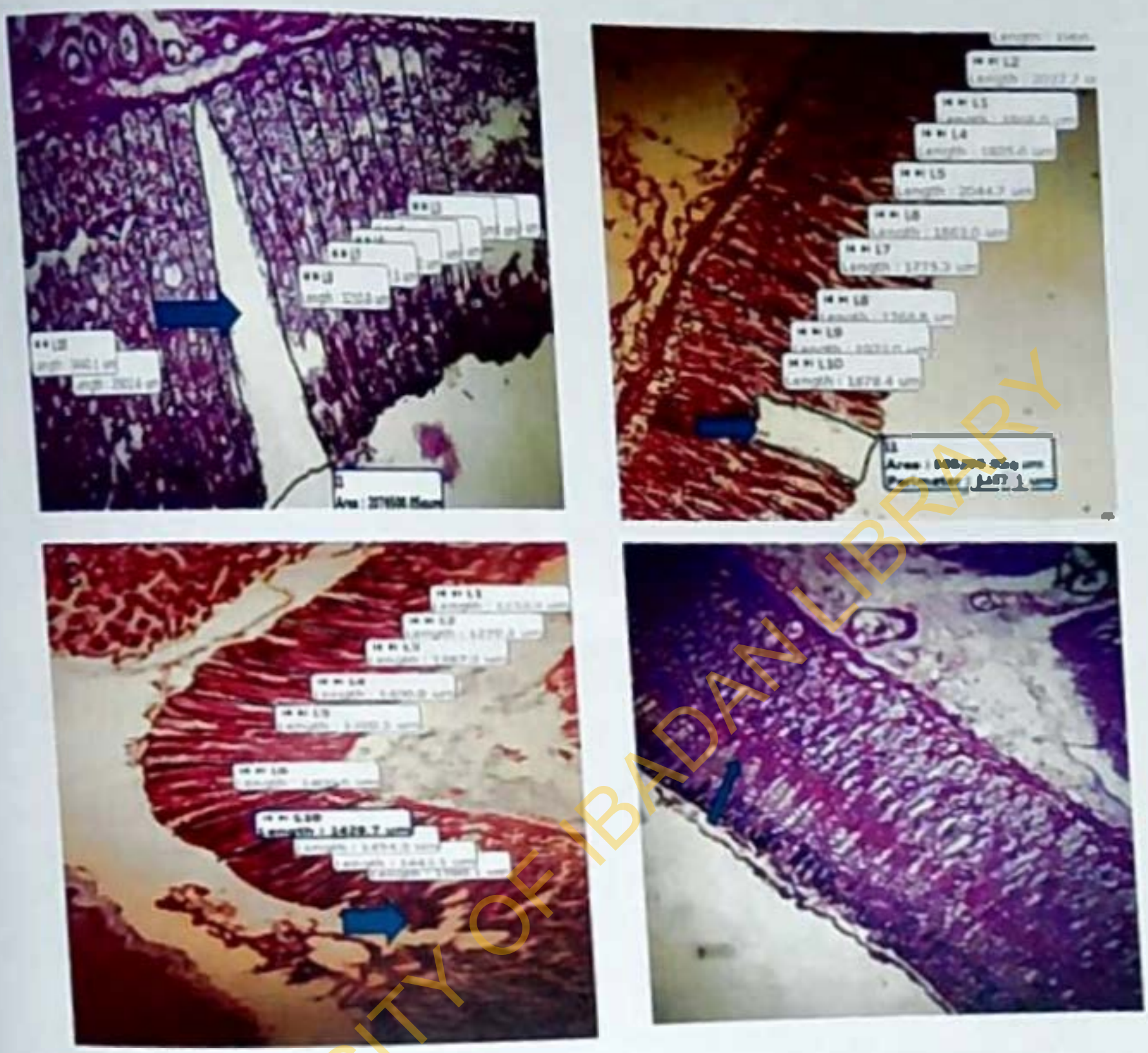

**Plate 9. Histological profile of the effect of risperidone on WIRS in control and treated animals' stomach section. H&E and PAS Staining, Mag. X100.**

A Control group – WIRS alone - severe ulcer

B Risperidone (0.1mg/kg) + WIRS - less severe ulcer

C Risperidone (0.3 mg/kg) + WIRS - mild ulcer

D Risperidone (0.5 mg/kg) + WIRS - no ulcer

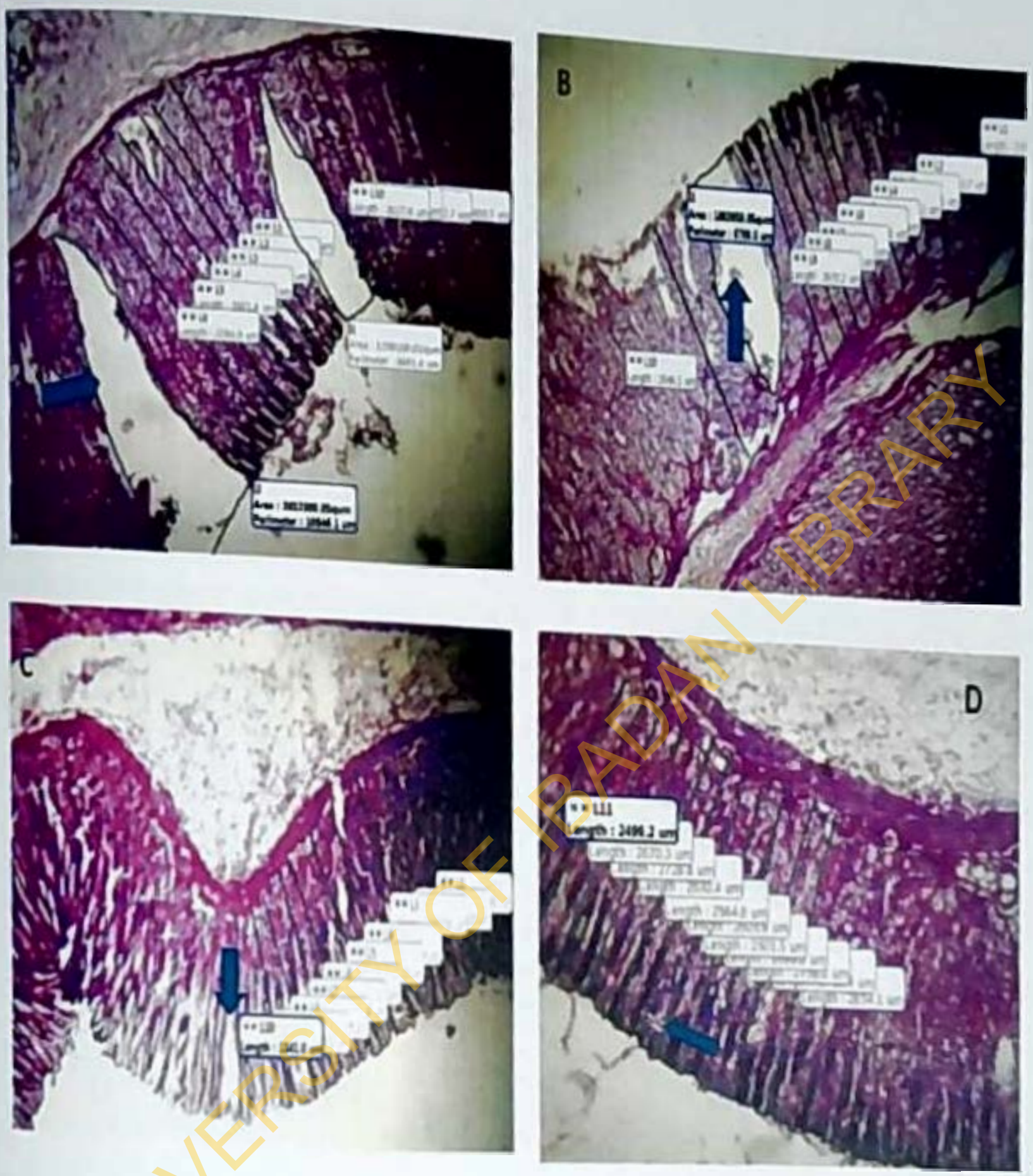




**Plate 10** Histological profile of the effect of risperidone on indomethacin-induced gastric ulceration on stomach section of control and treated animals. PAS Staining. Mag. X100

A Control group – indomethacin alone - very severe ulcer

B Risperidone (0.1mg/kg) + indomethacin - less severe ulcer

C Risperidone (0.3 mg/kg) + indomethacin - mild ulcer

D Risperidone (0.5 mg/kg) + indomethacin - lesser or no ulcer

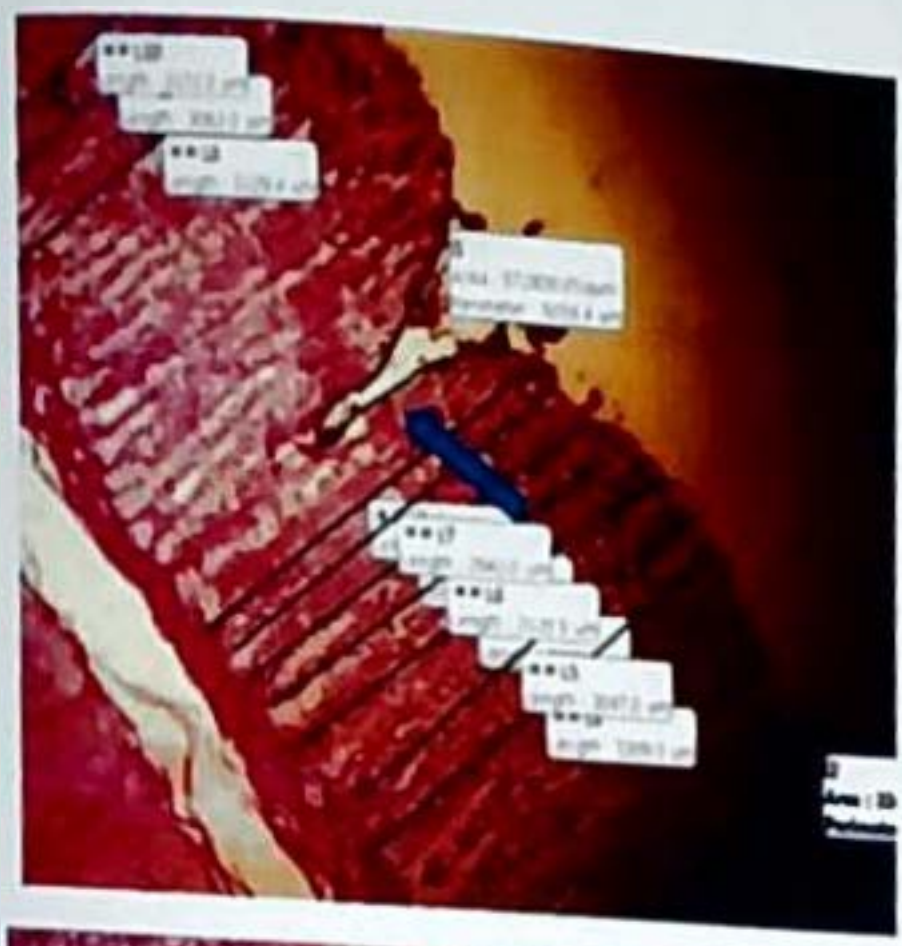

Plate 11. Histological profile of the effect of risperidone on starvation- induced ulcer on stomach section on control and treated animals. H&E and PAS Staining, Mag. X100. Arrow points to gastric lesion.

A Control group alone - very severe ulcer

B Risperidone (0.1mg/kg) - less severe ulcer

C Risperidone (0.3 mg/kg) - mild ulcer

D Risperidone (0.5 mg/kg) - less or no ulcer




Plate 12 Histological profile of the effect of risperidone on indomethacin (A), SC-560 and celecoxib combination (B), SC-560(C) and celecoxib (D) -induced gastric ulcer (x100).

A - Indomethacin

B - SC-560 and celecoxib combination

C - SC-560

D - Celecoxib

E - Risperidone

# CHAPTER FIVE

## 5.1 DISCUSSION

In this study, the anti-gastric ulcer effect of risperidone was investigated in male Wistar rats using water immersion restraint stress-, starvation- and indomethacin- ulcer models. In addition, the effect of risperidone on gastric acid secretion (GAS), gastric mucus secretion (GMS), gastric mucus cells count (GMCC) and gastric malonialdehyde (MDA) concentration, a measure of lipid peroxidation, were evaluated in rats. The roles of cyclooxygenase 1 and 2 inhibitors (SC 560 and Celecoxib) respectively on the action of risperidone were also investigated.

The three methods adopted to induce gastric ulcer in this work affirmed that risperidone at all doses used showed anti-ulcer activity. It was observed that risperidone significantly reduced mean ulcer scores, ulcer area ($\mu m^2$) and perimeter area ($\mu m$) in a dose-dependent fashion compared to control in WIRS model This is in agreement with an earlier study that reported risperidone-treatment alleviated the stress induced ulcers with increase in plasma levels of corticosterone, norepinephrine, glucose and total cholesterol using stress models (Sarieem and Singh 2011). These hormones are involved in stress. The degree of inhibition of ulceration increases with increase in dose of risperidone and this could be explained from the work of Byun *et al* 2007) that reported that corticosteroids is involved in stress-induced gastric ulceration in rats. Stress has been reported to be one of the aggressive factors and it underlies many other diseases apart from ulcers and is one of the most

commonly used methods to produce ulcer models (Brzozowski *et al.*, 2008; Kwiecień *et al.*, 2007). The ischemia that results from the stress has been reported to generate free radicals leading to oxidative damage and thus ulcer formation (Andrews *et al.*, 1992). It has also been reported that humans also present with stress (Kronfol *et al.*, 1984) and there are reports that showed gastrointestinal ulcers being present in patients with psychosis (Guldahl *et al.*, 1977). Other reports show that certain antipsychotics such as perospirone do have anti-ulcerative effects (Olden, 2005; Ishida- Tokuda *et al.*, 1996). From this study, risperidone in causing a decrease in ulcer formation and thus gastroprotection may be doing so by either reducing plasma corticosterone level or reducing the generation of free radicals from ischemia. This study is providing the premise that Risperidone is reducing the generation of free oxygen radicals to elicit anti-ulcer effects. This observation appears plausible because part of the work on MDA concentration showed that Risperidone reduces MDA levels in a dose-dependent fashion.

Risperidone significantly decreased the mean ulcer score, ulcer area $(\mu m)^2$, percentage ulcer area and perimeter ($\mu m$) of ulcer in a dose-dependent manner in Starvation-induced ulcer model compared to control. This model of ulcer induction are in agreement with Elegbe (1978) that reported that seven days period of starvation consistently produced gastric ulceration in rats, and also Robert and Nezamis (1958) who both reported that all the ulcers produced by this method occurred in the ruminal portion of the stomach of the rats. They observed that the mechanism(s) whereby prolonged starvation causes gastric ulceration may be due to an impairment of the gastric mucosal resistance to the acid content of the gastric juice or to an enhanced corrosive (i.e ulcerogenic) effect of the normal gastric juice. Even

though Elegbe (1978) and Robert and Nezamis (1958) reported the effect of the onset of ulceration beyond five (5) days of starvation, this work modified this model by examining the percentage ulcer area and perimeters of ulcer produced. This has been confirmed by the present study whereby ulcers are noticed as from day six (6), and death at day (7). Thus day six (6) was chosen as the reference. In the previous works, percentage ulcer area and perimeter of the ulcer were not calculated. In this study, the percentage ulcer area were noticed to decrease thus 24% (0.1 mg/kg), 11% (0.3 mg/kg) and then to 5.6% (0.5 mg/kg), while the perimeter of the ulcer (μm) decreased from 8430 in control to 3437 (0.1mg/kg), 2888 (0.3 mg/kg) and then to 2188 (0.5 mg/kg) as shown in Table 8. With this reduction in ulcer areas, risperidone is thus increasing or stabilizing gastric mucosal resistance to gastric corrosion and at the same time, risperidone reduces histamine $H_2$ stimulated gastric secretion. These two possible reasons may therefore support the anti-ulcer mechanism of Risperidone. Also this observation agreed with Hung and Neu *et al.*, (1997) who listed some factors that are likely to be involved in the formation of starvation-induced ulcer to include increase in gastric acid, increase in generation of free radicals, reduction in mucosal cytoprotective substances, reduction in mucosal blood flow and decrease in adenosine supply. Furthermore, hypoglycaemia caused by starvation may result in copious secretion of gastric acid. The gastric acid back-diffusion and free radicals, two offensive factors related to ulcer formation come into play (Davenpoll and Charvre, 1968). Since the integrity of the gastric mucosa is usually affected by both offensive and defensive factors, it is conceivable that under normal circumstances, the pure gastric juice is diluted and buffered by the swallowed food, water and saliva, mucus from the pyloric antrum and regurgitated duodenal

secretions, thus reducing or neutralizing its corrosive effect on the gastric mucosa. With prolonged starvation, the above neutralizing factors are present in insufficient amounts, the gastric content approximates to the pure fundic secretion in its corrosive properties. Under this condition, the mucosa succumbs and an ulcer is formed. Since nutrients such as glucose and amino acids are essentials for maintaining homeostatic functions of gastric cells, it is possible that deprivation of food leads to pathological changes of the gastric mucosa.

Indomethacin-induced ulcer model has been reported to be important in investigating the potential usefulness of anti-secretory and cytoprotective agents where the underlying pathophysiology involves gastric acid secretion and mucosal prostaglandin synthesis (Adinortey *et al.*, 2013). From this study risperidone in mitigating these factors significantly caused a dose-dependent significant decrease in ulcer scores, ulcer area $(\mu m)^2$, percentage of ulcer area and perimeter ($\mu m$) compared to the control in indomethacin-induced ulcer model and thus increase in the ulcer protection. This is similar to a study by Cao *et al.*, 2004 that reported pantoprazole sodium causing ulcer inhibition in aspirin-induced ulcer and that this effect is mainly due to acid inhibition. It is known that pure, undiluted gastric juice is an exceedingly corrosive fluid that can digest and destroy most living tissues, including the mucosa of the stomach. The increased acidity of the gastric juice enhances the effect of the deficiency of the gastric mucosa protective factors. Gastric mucosal injury is thought to result when aggressive luminal factors (gastric acid, NSAIDs etc.) overwhelm mucosal protective mechanism (Flemstrom *et al.*, 1982; Allen *et al.*, 1993). Risperidone may be mitigating some of these factors (gastric acid) as shown from this study. Though Swarnakar *et al.*, (2005) reported that indomethacin increases serum

cachexin or cachectin formerly known as Tumour Necrosis Factors alpha (TNF- α) an adipokine factor involved in systemic inflammation and mucosal thiobarbituric acid reactive substances (TBARS) at the ulcer site and is responsible for ulcerogeoesis. Risperidone reduced indomethacin-induced ulceration by reducing MDA, a metabolite of TBARS and NSAIDs have been shown to cause ulceration in the glandular portion of the stomach associated with increase in intragastric acidity or lowering of the pH. Thus, risperidone in reducing indomethacin-induced ulceration may be mitigating factors involved with indomethacin-induced ulceration.

The results obtained from the study on GAS showed that risperidone affected the normal gastric acid secretory response to histamine ($H_2$) and pentagastrin stimulation. With histamine ($H_2$) secretory response, there was no significant change in the basal secretion of gastric acid with the different doses of riperidone (0.1 mg/k, 0.3 mg/kg and 0.5 mg/kg). But when histamine (0.1 mg/g) was administered to these different risperidone pretreated animals, for 0.1 mg/kg there was complete removal of the normal response to histamine, while for 0.3 mg/kg and 0.5 mg/kg there was an initial rise followed by a decrease in response respectively. On pentagastrin secretory response, there were no remarkable changes on the basal secretion of gastric acid with the different doses of Risperidone (0.1 mg/kg, 0.3 mg/kg and 0.5 mg/kg). But, when pentagastrin (25 μg/kg) was injected, there was an initial increase in gastric acid secreted followed by significant decrease with the different doses of risperidone used as against the normal expectation whereby the basal secretion increased when pentagastrin was administered. From these observations, risperidone appears to cause a complete inhibition, blocking $H_2$ receptors, but with pentagastrin, there was partial inhibition indicating partial

inhibition of CCK-B receptors for gastrin. In both cases, GAS is significantly reduced, while there may be a significant depression in intracellular free $Ca^{2+}$. It is generally accepted that gastrin acts mainly by releasing histamine from the enterochromaffin-like (ECL) cells in the oxyntic mucosa, so also from actions on parietal cells where they are equally distributed (Prinz, 1994; Sandvik *et al.*, 1998). Furthermore, it has been reported that prostaglandins do exert inhibitory effects on parietal cells (Soll, 1986). From this study, apart from antagonizing $H_2$ and gastrin CCK-B receptors, risperidone may also be potentiating the effect of endogenous prostaglandins leading to inhibition of gastric acid secretion. Acetylcholine from nerve endings have been shown to acts by two possible pathways. First is the release of histamine from the ECL cells, which then stimulate acid production (Wollin, 1987) and secondly, by interaction with muscarinic ($M_3$) receptors on the oxyntic cells resulting in increase in intracellular calcium concentration (Wilkes *et al.*, 1991). Thus, the inability of risperidone to decrease acid secretion after carbachol administration may be due to its not effectively antagonizing the muscarinic receptors, even though histamine release has been effected.

From this work, there was significant increase in gastric mucus secretion (mg/g tissue) in the risperidone-treated rats compared to the control rats. Similarly, gastric mucus cells count (GMCC) showed a graded increase with increasing doses of risperidone. The possible explanation may likely be linked to potentiation of endogenous prostaglandins. Several other works are in support of this hypothesis, like that of Kerss *et al.*, 1982; McQueen *et al.*, 1983; Allen and Carroll, 1985; Allen, 1989 who all reported threefold increase in mucus layer thickness when topical prostaglandins and intravenous secretin were administered. The

study of Nishizaki *et al.*, (1994) also observed that cimetidine and prostaglandin $E_2$ ($PGE_2$) all increased mucus layer thickness by up to one-third. Part of this work showed that indomethacin caused gastric damage that is similar to that observed with the combination of COX-1 (SC- 560) and COX-2 (celecoxib) inhibitors. Risperidone reduced the gastric damage to the mucosal in both cases. The observation was that COX-1 and COX-2 given in combination provided the same results similar to that of indomethacin alone. When risperidone was given with each of these COX inhibitors, it produced a significant drop in ulcer scores. Suggestively, since there are two pathways of prostaglandins synthesis via COX-1 and COX-2, risperidone may therefore be involved in reversing the inhibitory effects of the inhibitors in any of the two pathways. This suppression of both COX-1 and COX-2 enzymes necessary for NSAID-induced gastric damage in the rat is consistent with a number of previous findings and in agreement with other studies that confirmed that inhibition of both COX-1 and COX-2 are required for induction of gastric mucosal damage (Wallace *et al.*, 2000; Grenzer *et al.*, 2001; Tanaka *et al.*, 2002). From the results, risperidone may be potentiating the effect of endogenous prostaglandins which has been reported to be involved in mucosal defense, stimulation of mucus and bicarbonate secretion (Wallace, 2008). Prostaglandin has also been reported to be important in ulcer healing and protection of gastric mucosal from digestive juice (Sano *et al.*, 1989; Wallace, 2008).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used as anti-inflammatory and analgesic agents, and indomethacin has been shown to produce higher gastric damage in rats when compared to other NSAIDs (Takeuchi *et al.*, 2005). Indomethacin caused gastric damage that is similar to that observed with the combination of

COX-1 and COX-2 inhibitors (SC-560 and Celecoxib). Risperidone reduced the gastric damage to the mucosal in both cases. The results also showed that separate administration of the selective COX-1 inhibitor (SC-560) and the selective COX-2 inhibitor (Celecoxib) with risperidone respectively did not cause any extensive damage to the stomach mucosa. The results obtained from the study is also in line with other reports that shows selective inhibition of COX-2 being associated with significantly less gastric erosion formation both in animals and humans (Masferrer *et al.*, 1994; Chan *et al.*, 1995; Simon *et al.*, 1999, Laine *et al.*, 1999). Wallace *et al.*, (2000) reported that the ability of NSAIDs to induce gastrointestinal injury depends on the inhibition of both COX-1 and COX-2. Thus, from the results obtained in this work, the adverse reaction of NSAIDs in the gastric mucosa may not be accounted for solely by the inhibition of COX-1, it also requires the inhibition of COX-2 as well. The result from the study showed that risperidone lessens the activity of COX-1 and COX-2 inhibitors as the damaging effect of NSAID's has been shown to reduce mucus gel thickness by impairing further the function of the barrier layer of the stomach mucosa (Ross *et al.*, 1981).

In the effect of risperidone on gastric MDA concentration, there was a significant dose-dependent decrease with 0.1mg/kg, 0.3 mg/kg and 0.5 mg/kg risperidone when compared to the control. However, it is known that indomethacin-induced gastric damage is reactive oxygen species (ROS) mediated lipid peroxidation (Naito *et al.*, 1998). Other reports have indicated that scavenging these free radicals may play an appreciable role in healing gastric ulcers (Loguercio *et al.*, 1993; Bandyopadhyay *et al.*, 1999). Risperidone has a strong reducing effect on lipid peroxidation and this could be a good reason for prevention of mucosal damage that may have been produced in absence of a reducing agent like

risperidone. Defensive factors such as mucus and reduced lipid peroxidation protect gastric mucosa against a variety of noxious agents-induced damages. There were significant increases in mucus secretion and cell counts respectively in the risperidone treated animals. The thiobarbituric acid reactive substance (TBARS) has been used as an indicator of lipid peroxidation or free radical scavenging activity in biological samples (Utely *et al.*, 1973). These radicals are also involved in acute mucosal ulceration induced by indomethacin (Wallace *et al.*, 1991). The product of these free radicals, the lipid peroxides, can elicit tissue inflammation (Link, 1993). The inhibition of cyclooxygenase (COX) enzymes induced by indomethacin leads to the depletion of endogenous prostaglandins thus leading to decrease of gastric mucus production (Nam *et al.*, 2005) and generation of reactive oxygen species, which are implicated in the pathogenesis of ulceration (Corfield *et al.*, 2001). Risperidone was found to scavenge reactive oxygen species as evident in its ability to decrease lipid peroxidation level. The important roles of oxygen-derived ROS and lipid peroxides (LPO) in acute gastric lesions, which are induced by nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, have been supported by experimental data (Jaimi and Devi, 2006; Ueno *et al.*, 2006). Similarly, indomethacin has been shown to produce damage via increasing mucosal MDA levels in gastric tissue (Odabasoglu *et al.*, 2006). Indomethacin causes gastric damage by not only inhibiting cytoprotective prostaglandin synthesis, but also by affecting antioxidant mechanisms, such as MDA. Thus, risperidone appears to exert its anti-ulcer effects by activation of antioxidant mechanisms in stomach tissues since antioxidant parameters have been shown to be reduced in stomach tissue damaged by indomethacin (Hassan *et al.*, 1991). The roles of toxic oxygen radicals has also been reported

to be involved in indomethacin- induced gastric damage as was determined in ulcerogenesis in a study by Naito *et al.*, (1998). This study showed that risperidone significantly prevented the negative effect of indomethacin on gastric MDA levels at all doses used. Increased levels of reactive oxygen species (ROS) are indicated in the mechanism of both stress and indomethacin-induced gastric damage (Itoh and Guth, 1985). Excessive production of MDA and other reactive radicals cause oxidative damage which is represented by measuring lipid peroxidation levels (Peralta et al., 2001). Lipid peroxidation is an important reason for cell membrane damage; MDA is the end product of lipid peroxidation and is used to determine lipid peroxidation levels (Nielsen *et al.*, 1997). In this work, gastric MDA concentration levels decreased with increasing risperidone treatment.

Histology revealed reduced gastric ulceration in the entire risperidone pretreated groups compared to the control. Histological studies confirm these results by showing the occurrence of mucosal ulceration and the damage of epithelial and lamina propria cells in the control animals compared to the risperidone treated groups that showed a dose-dependent amelioration of ulcer. The various histological plates confirmed the strong role of risperidone at the microlevel, that it can be effective in the complete prevention and healing of gastric ulcer.

# CHAPTER SIX

## 6.1 CONCLUSION

The result of this study showed that the mechanisms of the anti-gastric ulcer activity risperidone include reducing ulcer scores through potentiating prostaglandins secretion. It also includes reducing histamine and pentagastrin stimulation of gastric acid secretion through the blocking of $H_2$ and CCK-B receptors for gastrin. Risperidone decreases lipid peroxidation (MDA concentration) level by manifesting an increased anti-oxidant activity, increased gastric mucus secretion and gastric mucus cells count. All these effects contribute to its gastroprotective, anti-secretory activity and up-regulating activity on cyclooxygenase enzymes.

## 6.2 RECOMMENDATION

This work reveals that risperidone, an anti-psychotic drug used in the management of psychotic and schizophrenic symptoms may be beneficial for the management of patients presenting with gastric ulcer.

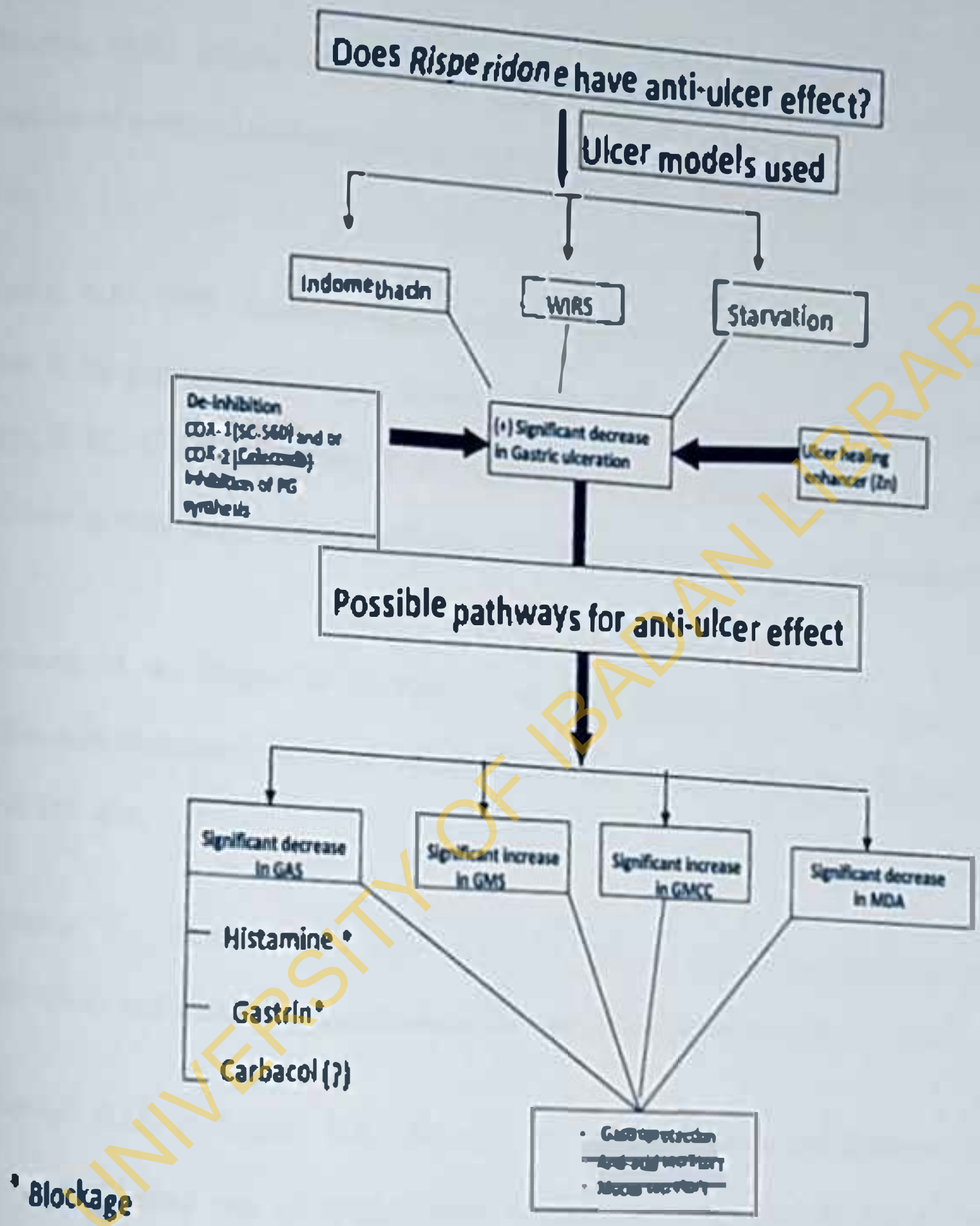


Figure 27 Proposed mechanisms of the anti-gastric ulcer effect of risperidone.

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# APPENDIX

## STARVATION INDUCED- ULCER

| animal | Control | 0.1mg/kg | 0.3mg/kg | 0.5mg/kg |
|---|---|---|---|---|
| 1. | 6.0 | 4.0 | 2.0 | 1.0 |
| 2. | 5.0 | 3.0 | 1.0 | 0.5 |
| 3. | 4.0 | 4.0 | 3.0 | 0.5 |
| 4. | 7.0 | 3.0 | 3.0 | 1.0 |
| 5. | 6.0 | 3.0 | 2.0 | 2.0 |
| 6. | 5.5 | 4.0 | 2.0 | 1.5 |
| 7. | 6.0 | 4.0 | 1.0 | 1.0 |
| 8. | 5.0 | 3.0 | 1.0 | 1.5 |
| Mean | 5.563 | 3.500 | 1.875 | 1.125 |
| SEM | 0.3196 | 0.1890 | 0.2950 | 0.1830 |

**INDOMETHACIN INDUCED ULCER**

| Animals | Control | 0.1mg/kg | 0.3mg/kg | 0.5mg/kg |
|---|---|---|---|---|
| 1. | 6.0 | 5.5 | 2.0 | 1.0 |
| 2. | 6.5 | 6.0 | 3.0 | 2.0 |
| 3. | 5.0 | 5.0 | 2.5 | 1.0 |
| 4. | 6.0 | 4.5 | 2.0 | 1.5 |
| 5. | 7.0 | 4.0 | 1.5 | 1.5 |
| 6. | 5.5 | 5.0 | 1.5 | 2.0 |
| 7. | 7.5 | 4.0 | 2.0 | 0.5 |
| 8. | 8.0 | 5.5 | 1.5 | 1.0 |
| Mean | 6.438 | 4.938 | 2.000 | 1.313 |
| SEM | 0.3590 | 0.2577 | 0.1890 | 0.1875 |

## WATER IMMERSION RESTRAINT INDUCED –ULCER (WIRS)

| Animal | Control | 0.1 mg/kg | 0.3mg/kg | 0.5mg/kg |
|---|---|---|---|---|
| 1. | 5.5 | 5.0 | 2.0 | 1.5 |
| 2. | 5.0 | 4.5 | 2.5 | 1.0 |
| 3. | 6.0 | 4.0 | 3.0 | 2.5 |
| 4. | 5.0 | 5.0 | 1.5 | 2.0 |
| 5. | 7.0 | 3.5 | 1.5 | 1.5 |
| 6. | 6.5 | 3.5 | 2.0 | 1.0 |
| 7. | 6.0 | 4.0 | 3.0 | 2.0 |
| 8. | 7.5 | 2.5 | 2.5 | 2.5 |
| Mean | 6.063 | 4.000 | 2.250 | 1.750 |
| SEM | 0.3196 | 0.2988 | 0.2113 | 0.2113 |

**PENTAGASTRIN INDUCED GASTRIC ACID SECRETION (GAS) IN RISPERIDONE TREATED RATS (mean ± SEM)**

| Time (mins.) | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 |
|---|---|---|---|---|---|---|---|---|
| Control | 0.92 ± 0.03 | 1.05 ± 0.08 | 1.43 ±0.14 | 1.72 ±0.21 | 2.93 ±0.081 | 3.35 ±0.13 | 3.67 ±0.10 | 3.27 ±0.05 |
| 0.1mg/kg | 0.72 ±0.06 | 0.90 ±0.07 | 1.02 ±0.07 | 1.53 ±0.06 | 1.88 ±0.02 | 1.52 ±0.05 | 1.50 ±0.05 | 1.35 ±0.04 |
| 0.3mg/kg | 0.73 ±0.07 | 0.87 ±0.06 | 0.88 ±0.12 | 1.35 ±0.08 | 1.83 ±0.03 | 1.38 ±0.07 | 1.15 ±0.04 | 1.07 ±0.10 |
| 0.5mg/kg | 0.58 ±0.05 | 0.53 ±0.03 | 0.80 ±0.05 | 1.15 ±0.11 | 1.72 ±0.07 | 1.45 ±0.12 | 1.18 ±0.08 | 1.12 ±0.03 |

**CARBACHOL INDUCED GASTRIC ACID SECRETION (GAS) IN RISPERIDONE TREATED RATS (mean ± SEM)**

| Time (mins.) | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 |
|---|---|---|---|---|---|---|---|---|
| Control | 0.37 ± 0.06 | 0.43 ± 0.07 | 0.52 ±0.13 | 0.65 ± 0.16 | 2.47 ± 0.14 | 2.72 ± 0.06 | 2.67 ±0.07 | 2.85 ±0.08 |
| 0.1mg/kg | 0.57 ± 0.07 | 0.65 ±0.08 | 0.55 ±0.06 | 0.73 ±0.07 | 2.12 ±0.08 | 2.62 ±0.06 | 2.63 ± 0.07 | 2.63 ±0.06 |
| 0.3mg/kg | 0.60 ± 0.04 | 0.75 ±0.07 | 0.65 ±0.06 | 0.83 ±0.07 | 2.42 ± 0.08 | 2.68 ± 0.07 | 2.67 ±0.06 | 2.53 ±0.12 |
| 0.5mg/kg | 0.47 ±0.08 | 0.53 ±0.11 | 0.60 ± 0.11 | 0.62 ±0.07 | 2.40 ± 0.10 | 2.30 ± 0.08 | 2.30 ±0.05 | 2.40 ± 0.06 |

## GASTRIC MUCUS SECRETION (GMS)

| Rats | Control | 0.1mg/kg | 0.3mg/kg | 0.5mg/kg |
|---|---|---|---|---|
| 1. | 0.652 | 1.400 | 1.200 | 1.750 |
| 2. | 0.661 | 1.600 | 1.500 | 0.610 |
| 3. | 0.600 | 1.000 | 1.700 | 0.520 |
| 4. | 0.400 | 0.780 | 1.100 | 1.170 |
| 5. | 0.590 | 1.100 | 1.100 | 1.500 |
| 6. | 0.580 | 0.673 | 1.700 | 1.700 |
| 7. | 0.593 | 1.130 | 1.480 | 1.870 |
| 8. | 0.697 | 0.780 | 0.710 | 1.990 |
| Mean | 0.5966 | 1.058 | 1.311 | 1.389 |
| SEM | 0.03171 | 0.1137 | 0.1217 | 0.2001 |

## GASTRIC MUCUS CELLS COUNT (GMCC)

| Animal | Control | 0.1mg/kg | 0.3mg/kg | 0.5mg/kg |
|---|---|---|---|---|
| 1. | 103.0 | 131.6 | 130.6 | 129.4 |
| 2. | 105.8 | 117.0 | 140.4 | 115.0 |
| 3. | 87.8 | 123.0 | 116.2 | 136.0 |
| 4. | 97.0 | 94.2 | 128.2 | 140.4 |
| 5. | 108.0 | 107.4 | 123.0 | 138.2 |
| 6. | 97.2 | 136.2 | 129.2 | 137.6 |
| 7. | 128.2 | 130.0 | 130.0 | 114.4 |
| 8. | 99.6 | 129.8 | 131.4 | 123.4 |
| Mean | 103.3 | 121.2 | 128.6 | 129.3 |
| SEM | 4.182 | 5.044 | 2.459 | 3.726 |

**TABLE OF EFFECT OF RISPERIDONE ON INDOTHEMACIN, CYCLOOXYGENASE 1 (SC 560) AND 2 (CELECOXIB) INHIBITORS INDUCED ULCER**

| Rats | Indomet | SC-560 + Cel | Risp. +Indomet | Risp.+ SC-560 + Cel | Risp. + SC-560 | Risp. + Cel |
|---|---|---|---|---|---|---|
| 1 | 6.2 | 5.0 | 1.5 | 2.0 | 0.0 | 0.0 |
| 2 | 4.5 | 4.5 | 2.5 | 1.5 | 0.0 | 0.5 |
| 3 | 5.5 | 5.5 | 2.0 | 2.5 | 0.5 | 0.0 |
| 4 | 5.0 | 6.0 | 1.5 | 1.0 | 0.5 | 0.5 |
| 5 | 3.5 | 3.5 | 2.0 | 1.5 | 0.0 | 0.0 |
| 6 | 4.0 | 2.5 | 1.0 | 1.5 | 0.0 | 0.5 |
| 7 | 3.6 | 3.0 | 1.5 | 2.0 | 0.5 | 0.0 |
| 8 | 4.2 | 4.0 | 1.0 | 1.0 | 0.0 | 0.0 |
| Mean | 4.6 | 4.3 | 1.6 | 1.6 | 0.2 | 0.2 |
| SEM | 0.3 | 0.4 | 0.2 | 0.2 | 0.1 | 0.1 |

Indometh – Indomethacin (10mg/kg)

Cele - Celecoxib (15mg/kg)

Risp - Risperidone (0.5mg/kg)

## MALONDIALDEHYDE CONCENTRATION (MDA)

| Animal | Control | 0.1mg/kg | 0.3mg/kg | 0.5mg/kg |
|---|---|---|---|---|
| 1. | 0.2481 | 0.1987 | 0.1987 | 0.1077 |
| 2. | 0.2340 | 0.1763 | 0.2026 | 0.0949 |
| 3. | 0.2885 | 0.1647 | 0.1436 | 0.1347 |
| 4. | 0.2289 | 0.2128 | 0.1705 | 0.1090 |
| 5. | 0.2692 | 0.2218 | 0.1744 | 0.1000 |
| 6. | 0.2949 | 0.1795 | 0.1897 | 0.1039 |
| 7. | 0.2410 | 0.1859 | 0.1827 | 0.0885 |
| 8. | 0.2500 | 0.2115 | 0.2051 | 0.1115 |
| **Mean** | **0.2568** | 0.1939 | 0.1834 | 0.1063 |
| SEM | 0.008742 | 0.007199 | 0.007252 | 0.004883 |


A Photograph of Risperidone tablets

Illustration of the Effect of Risperidone on Water immersion restraint stress induced gastric ulceration