CHAPTER ONE

INTRODUCTION

The gastrointestinal tract is one of the most important systems in the body. This is because it is the only system in the body responsible for providing nutrients and other biological molecules needed for growth, development and sustenance of the organism. All ingested food, condiments, drugs and other substances enter the stomach after leaving the mouth and oesophagus. There in the stomach, these substances spend variable amounts of time during which they are digested and/or absorbed. The process of digestion and absorption makes the stomach to be susceptible to normal "wear and tear". However, there are some mechanisms found in the stomach that protect it from these assaults and minimize the damage that would otherwise have occurred.

One of the sources of assault on the gastrointestinal tract (and the stomach in particular) is environmental toxicants e.g. smoke, smog, fog, dust, heavy metals etc. Examples of some heavy metals that contaminate the environment are lead, cadmium, mercury, chromium, arsenic and vanadium. These environmental toxicants come from some industries such as lead smelter plants, battery manufacturing/recycling plants, tannery, agricultural production, metal mining industries and even small scale industries such as artisanal welding and joinery.

Environmental lead contamination is common in industrialized societies and has serious implications for sustenance of good health (Alperstein *et al*, 1991). Lead is a poisonous metal which has caused considerable concern to the health of neonates and developing fetus (Donald *et al*, 1986). Lead has many adverse health effects, including toxicity of the hematopoietic, renal, endocrine and skeletal systems. The central nervous system (CNS) is however the main target of lead toxicity. Lead toxicity occurs from low level exposure to various environmental

and occupational sources including food, air and water (Peraza *et al*, 1998). Many of the experimental studies on low levels of lead exposure/treatment have been performed using the laboratory rat. These studies have demonstrated a variety of effects such as a delayed cortical synaptogenesis (McCauley *et al*, 1982), exploration and locomotor activity (Crofton *et al*, 1980), and decreased packed cell volume (Sharma and Pandey, 2010). Previous research on the effects of micronutrients on toxicity caused by various environmental pollutants such as lead and cadmium has produced growing evidence in the field of micronutrients intake and corresponding reduction of heavy metal toxicity. Micronutrients can reduce toxicity of heavy metals by interacting with the metal at its primary site of action. Examples of this type of interaction include the effects of calcium on lead, phosphate on arsenate, and zinc on cadmium (Peraza *et al*, 1998). Lead induced oxidative stress contributes to the pathogenesis of lead poisoning by disrupting the delicate prooxidant/antioxidant balance that exists within living cells. Production of reactive oxygen species (ROS) is increased after lead treatment in *in vitro* studies. *In vivo* studies suggest that lead exposure causes generation of ROS and alteration of antioxidant defense systems in animals and occupationally exposed workers (Hsu and Guo, 2002). Exposure to lead caused a significant inhibition of blood aminolevulinic acid dehydratase, an important enzyme in the haem synthesis pathway. Lead exposure also led to a pronounced depletion of brain GSH contents, super oxide dismutase activity, an increase in thiobarbituric acid reactive substances and activity of glutathione S-transferase. Specific activities of membrane-bound enzymes, such as acetylcholinesterase and monoamine oxidase were significantly inhibited by lead exposure. These biochemical changes were correlated with increased uptake of lead in blood and soft tissues (Saxena and Flora, 2004).

A variety of disorders affect the gastrointestinal tract. Some of them are ulcers, gastritis, colitis, Crohn's disease, etc. A gastric ulcer is an erosion of the top layer of cells from the stomach wall leaving the second and subsequent underlying layers of cells exposed without protection (William, 1977). Also, an ulcer may be defined as a deep defect in the esophageal, gastric, duodenal and/ or intestinal wall penetrating the entire mucosal thickness and the muscularis mucosa (Tarnawski, 2000). Peptic ulcer is one of the most prevalent disorders of the gastrointestinal tract (Poonam *et al.*, 2003). Healing of peptic ulcers involves several processes such as granulation tissue formation, re-epithelialization, tissue remodeling, cell proliferation, cell division, cell hypertrophy, clot formation, phagocytosis, fibroblast migration, collagen deposition, vascular remodeling, mucosal regeneration, reconstruction of gastric glands, angiogenesis, muscle restoration, etc. (Tarnawski, 2000).

Lead (Latin: *Plubium*) is one of the oldest metals known. The atomic weight of lead is 207. It is one of seven metals used in the ancient world. The others are gold, silver, copper, iron, tin, and mercury. Its low melting point of 327° C coupled with its easy castability, softness and malleability make lead and lead alloys especially suitable for a wide range of cast products. Such products include battery grids and terminals, counterweights, plumbing components and type metal (Polyanskiy, 1986). With a specific gravity of about 11.35 grams per cubic centimeter, lead is one the densest metals, this makes it a good shield against X-rays and gamma radiation (Polyanskiy, 1986). Lead is widely distributed all over the world in the form of Lead sulfide (Galena). Lead ranks about 36th in natural abundance among elements in Earth's crust. Lead is one of the most dangerous heavy metals that contaminate the environment (Goyer and Rhyme, 1973).

In man, routes of exposure to lead include ingestion with food, water and other products and via inhalation (Anetor *et al*, 2003). Substantial exposure of individuals to lead also occurs in the workplace environment (Al Saleh, 1999). Lead poisoning (also known as plumbism) is a medical condition caused by increased levels of lead in the body. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, reproductive and nervous systems

Symptoms of lead poisoning include headache, anemia, paralysis, weakness, irritability, reduced intelligence, delayed motor development, impaired memory, hearing problems and in severe cases seizures, coma, and death (Ronis *et al.*, 2000). Lead poisoning also affects the gastrointestinal tract, causing abdominal pain, constipation, colic, diarrhea, poor appetite, etc.

It is known that exposure to certain agents such as environmental pollutants (e.g. cadmium, mercury, asbestos, lead etc.) will affect the formation of ulcers directly or indirectly. Lead has been shown previously to enhance the formation of experimentally induced ulcers (Olaleye *et al* 2006). Lead has also been shown to increase oxidative stress in the gastric mucosa of rats (Olaleye *et al.*, 2007).

However to the best of our knowledge, no work has been done on the effects of chronic lead exposure on healing of experimental gastric ulceration in the gastrointestinal tract of rats. The aim of the present study therefore, was to determine the effects of chronic lead exposure on healing of gastric ulcer in a rat model. The mechanisms of action of the observed changes will also be investigated.

Specifically, the aims and objectives of this study were to:

1. Investigate the effects of chronic lead exposure on healing of gastric ulceration in rats.

2. Elucidate the possible mechanism(s) of action of chronic lead exposure on healing of gastric ulceration in rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Stomach

The stomach is a muscular, hollow, dilated part of the digestive system. It functions as an important organ of the digestive tract in some animals, including vertebrates, echinoderms, insects (mid-gut), and molluscs. It is involved in digestion of food, following mastication.

The stomach is located between the esophagus and the small intestine. It secretes several protein-digesting enzymes and hydrochloric acid to aid in food digestion, (sent to it via the oesophagus). The partially digested food (chyme) is then sent to the duodenum.

The word *stomach* is derived from the Latin *stomachus* which is derived from the Greek word *stomachos*.

Although the precise shape and size of the stomach varies widely among different vertebrates, the positions of the oesophageal and duodenal openings remain relatively constant. As a result, the organ always curves somewhat to the left before curving back to meet the pyloric sphincter. However, some animals like hagfish and lungfish do not have stomachs. These animals consume diets that either requires little storage of food, or no pre-digestion with gastric juices, (Romer *et al.*, 1977).

The gastric mucosal lining is usually divided into two regions, an anterior portion lined by fundic glands, and a posterior with pyloric glands. Cardiac glands are unique to mammals. The distributions of these glands vary between species. Ruminants have a complex stomach, the first three chambers of which are all lined with oesophageal mucosa (Romer *et al.,* 1977).

Sections of the stomach

The stomach is divided into four major sections, each of which has different cells and functions. The sections are in cranio-caudal direction as follows: cardia, fundus, body and pylorus. 1. Cardia: this is the first part of the stomach. Here the contents of the oesophagus empty into the stomach via the lower oesophageal sphincter. 2. Fundus: this is formed by the upper curvature of the stomach. It is the dilated superior part next to the cardia. 3. Body or Corpus: this is the main, central region of the stomach. Most of the food in the organ are found in this portion. It is located between the fundus and the pyloric antrum 4. Pyloric part: this is the lower section of the organ that facilitates emptying the contents into the small intestine. It is separated from the duodenum by the pyloric sphincter (Moore et al., 2010).

Blood supply of the stomach

The lesser curvature of the stomach is supplied by the right gastric artery inferiorly, and the left gastric artery and posterior gastric artery superiorly, which also supplies the cardiac region. The greater curvature is supplied by the right gastro-omental artery inferiorly and the left gastro-omental artery superiorly. The fundus of the stomach, and also the upper portion of the greater curvature, is supplied by the short gastric artery which arises from splenic artery. Branches from the gastroduodenal artery also supply the lower parts of the stomach (Moore et al., 2010).

Cross section of the stomach

Like the other parts of the gastrointestinal tract, the stomach walls are made of the following layers, from inside to outside:

Mucosa: this is the first main layer. It consists of the epithelium and the lamina propria (composed of loose connective tissue), with a thin layer of smooth muscle called the muscularis mucosae separating it from the submucosa beneath.

Submucosa: This layer lies over the mucosa and consists of fibrous connective tissue, separating the mucosa from the next layer. The Meissner's plexus is in this layer (submucosal plexus). ***Muscularis Externa:*** located over the submucosa, the muscularis externa in the stomach differs from that of other GI organs in that it has three layers of smooth muscles instead of two. They are: 1. ***Inner oblique layer****:* This layer is responsible for creating the motion that churns and physically breaks down the food. It is the only layer of the three which is not seen in other parts of the digestive system. The antrum has thicker muscle cells in its walls and performs more forceful contractions than the fundus. 2. ***Middle circular layer****:* At this layer, the pylorus is surrounded by a thick circular muscular wall which is normally tonically constricted forming a functional pyloric sphincter, which controls the movement of chyme into the duodenum. This layer is concentric to the longitudinal axis of the stomach. *3.* ***Outer longitudinal layer.*** This is the outermost layer of muscles in the stomach. The fibers run along the length of the stomach.

Auerbach's plexus (myenteric plexus): is found between the outer longitundinal and the middle circular layers. It is responsible for the innervation of both muscle layers causing peristalsis and mixing movements.

Serosa: This layer is over the muscularis externa, consisting of layers of connective tissue. It is continuous with the peritoneum (Moore *et al*., 2010).

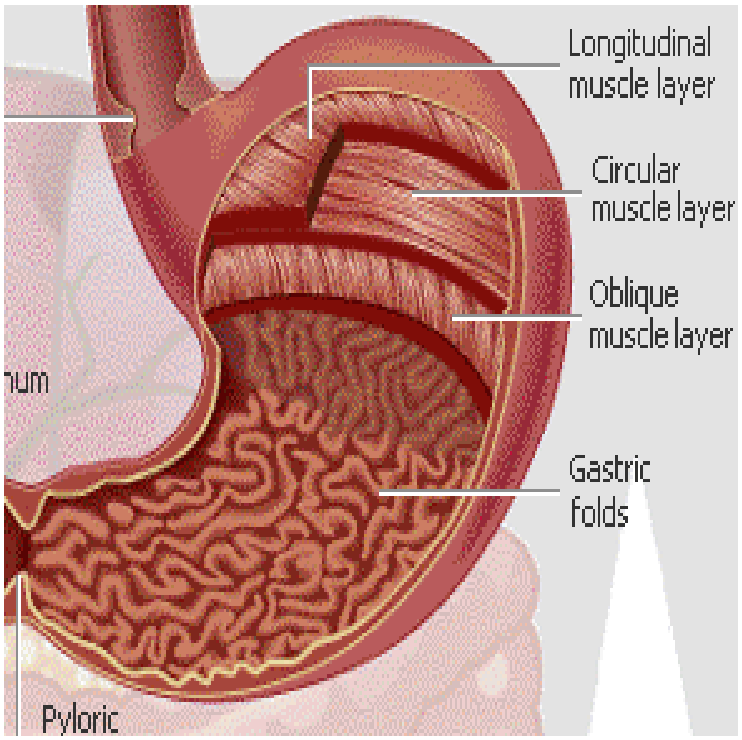

Figure 1a: Gross anatomical structure of the stomach.

Adapted from: Root, Walter S. "Stomach." Microsoft Student 2009. Redmond, WA: Microsoft Corporation.

Hormones involved in regulation of gastric activity

Virtually all aspects of gastric function are controlled by hormones. The various movements (motility) and flow of chemicals (secretion) into the stomach are controlled by both the nervous system and by the various hormones of the digestive system. Some of the hormones that control gastric activities are: gastrin, cholecystokinin, secretin, gastric inhibitory peptide, enteroglucagon, etc.

Gastrin: The hormone *gastrin* is causes an increase in the secretion of HCl from the parietal cells, and pepsinogen from chief cells in the stomach. It also causes increased motility in the stomach. Gastrin is produced and released by G-cells in the stomach in response to distention of the antrum, and presence of digestive products (especially large quantities of incompletely digested proteins). It is inhibited by a pH less than 4 (acid), as well as the hormone called somatostatin.

Cholecystokinin (CCK): is secreted by I cells in the mucosa of the upper small intestine. It exists in several forms such as CCK-58, CCK-39, CCK- 33, etc. It has most effect on the gall bladder causing gall bladder contractions. It also decreases gastric emptying by inhibiting motility, and increases release of pancreatic juice which is alkaline and neutralizes the chyme.

Secretin: secretin was discovered in 1902 by two scientists- Bayliss and Starling. It is produced by S cells that are located deep in the glands of the mucosa of the upper portion of the small intestine. It has most of its effects on the pancreas, but will also reduce acid secretion in the stomach and cause contraction of pyloric sphincter.

Gastric inhibitory peptide (GIP): this hormone is made up of 42 amino acid residues. It is produced by K cells in the mucosa of the duodenum and jejunum. Its secretion is stimulated by

glucose and fat in the duodenum. In large doses, it decreases both gastric acid secretion and motility.

Vasoactive intestinal polypeptide*:* it contains 28 amino acid residues. Its half life in blood is 2 minutes. It decreases gastric acid secretion (Barret *et al*., 2012).

Role of stomach in digestion of food

Ingested food from the mouth enters the stomach through the oesophagus via the oesophageal sphincter. The stomach secretes proteases (protein-digesting enzymes such as pepsin) and hydrochloric acid. The acid kills or inhibits bacteria and provides the acidic pH of 2.0 for the proteases to digest protein. Food is churned by the stomach through muscular contractions of the wall called in a process called peristalsis (Gore and Levine, 2007). Gradually, the boluses of food are converted into chyme (partially digested food). Chyme slowly passes through the pyloric sphincter into the duodenum, where absorption of nutrients continues. Depending on the quantity and contents of the meal, the stomach will digest the food into chyme within a few hours.

Two sphincters keep the contents of the stomach contained. They are the esophageal sphincter which separates the stomach from the oesophagus above. The other is the pyloric sphincter dividing the stomach from the duodenum below.

In adult humans, the stomach has a relaxed, near empty volume of about 45 mL. Because it is a distensible organ, it normally expands to hold about one litre of food, (Sherwood, 1997) but can hold as much as two to three litres. The stomach of a newborn human baby will only retain about 30 mL.

Stomach as Nutrition Sensor

The stomach can "taste" sodium glutamate using glutamate receptors (Uematsu *et al*., 2009). This information is passed to the lateral hypothalamus and limbic system in the brain as a palatability signal through the vagus nerve (Uematsu *et al*., 2010). The stomach can also sense independently to tongue and oral taste receptors glucose (De Araujo *et al.,* 2008), carbohydrates (Perez *et al*., 1996), proteins, and fats (Ackroff et al., 2005). This allows the brain to connect the nutritional value of foods to their tastes (Uematsu *et al.*, 2010).

Absorption in the stomach

Normally, absorption of nutrients is mainly a function of the small intestine. However, some absorption of certain small molecules does occur in the stomach. These molecules include: water, (if the body is dehydrated), medication (e.g. Aspirin), Amino acids, Vitamin B12, etc.

Gastric juice secretion

The stomach contains many types of glands. These glands secrete different chemicals involved in protection of the stomach tissue, and digestion of different food materials. In the pyloric and cardiac regions, the glands secrete mucus. In the body of the stomach and fundus, the parietal cells secrete hydrochloric acid and intrinsic factor. The chief cells secrete pepsinogens. The surface mucous cells secrete mucus and bicarbonate (HCO_3-). The enterochromaffin-like cells secrete histamine. Taken together, the cells of the gastric glands secrete over 2500mL of gastric juice daily. The juice contains different substances such as cations: Na^+, K^+, Mg^{2+}, H^+; anions: Cl-, HPO_4^{2-} , SO_4^{2-} ; pepsins, lipase, mucus, intrinsic factor, water, gastrin, etc. The hydrochloric acid secreted by the stomach kills ingested bacteria, aids protein digestion stimulates flow of bile and pancreatic juice.

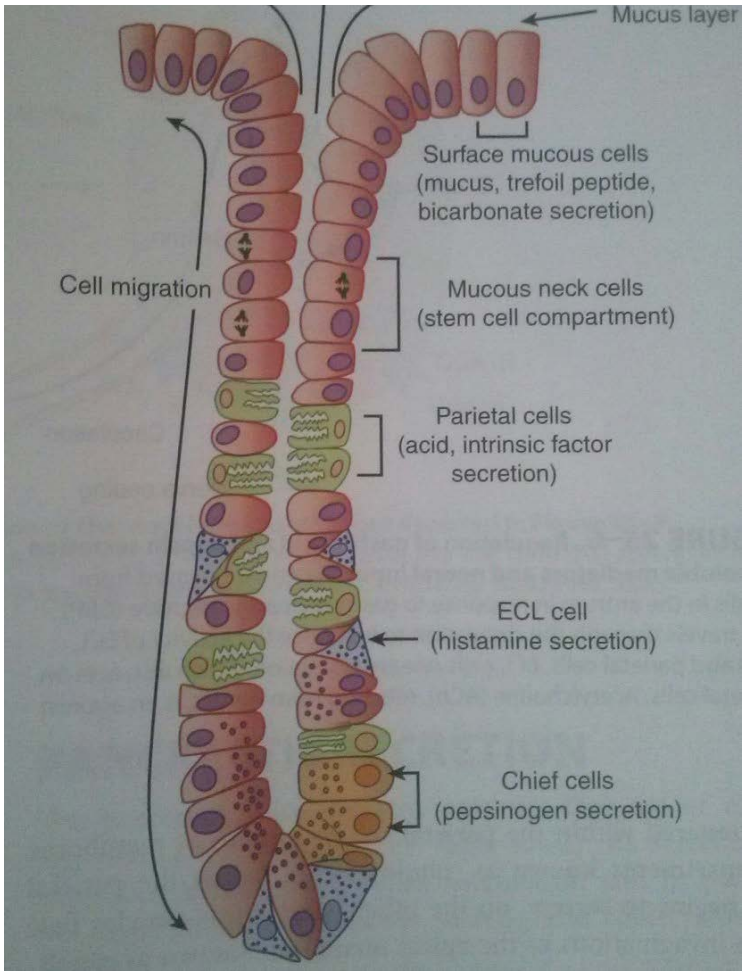

Figure 1b: Structure of a gastric gland showing different cell types and their secretions (Barrett *et al*., 2012)

The mucus and bicarbonate (HCO_3-) help to protect the stomach from the acid and digestive enzymes so that the stomach itself is not digested. The tight junctions between the epithelial cells are part of the mucosal barrier that protects the gastric epithelium from damage (Ganong, 1995). Substances that tend to disrupt the barrier and cause gastric irritation which may lead to ulceration are ethanol, bile salts, vinegar, aspirin, etc. prostaglandins on the other hand stimulate mucus secretion and thus help to protect the gastric epithelium. The hydrochloric acid produced by parietal cells has a pH of 0.87. The acid is formed as follows: Water combines with carbon dioxide (CO_2) to form H_2CO_3 in the parietal cells. The reaction is catalyzed by carbonic anhydrase. The H_2CO_3 formed dissociates into HCO_3^- and H^+. The HCO_3^- is exchanged for Cl^- by an antiport located on the basolateral membrane into the interstitial fluid. The H^+ is exchanged for K^+ at the apical membrane through H^+-K^+ transporter. Some Cl^- and K^+ diffuse into the gastric lumen. At the basolateral membrane, Na^+ is also exchanged for K^+. Finally, the H^+ and Cl^- combine in the gasric lumen to form HCl (Ganong, 1995). Acid secretion is stimulated by histamine via H_2 receptors, by acetylcholine via M_1 muscarinic receptors and gastrin via gastrin receptors (Ganong, 1995).

Diseases of the stomach

Some of the diseases affecting the stomach are gastric ulcer, gastritis and stomach cancer. Most diseases of the stomach are due to imbalance in the delicate homeostatic mechanisms that control epithelial cell proliferation/destruction, gastric acid secretion, digestive enzymes production and the mucosal defense factors. Mucosal defense factors include bicarbonate and protective mucus. Presence of the bacterium *Helicobacter pylori* also plays a key role in the pathophysiology of

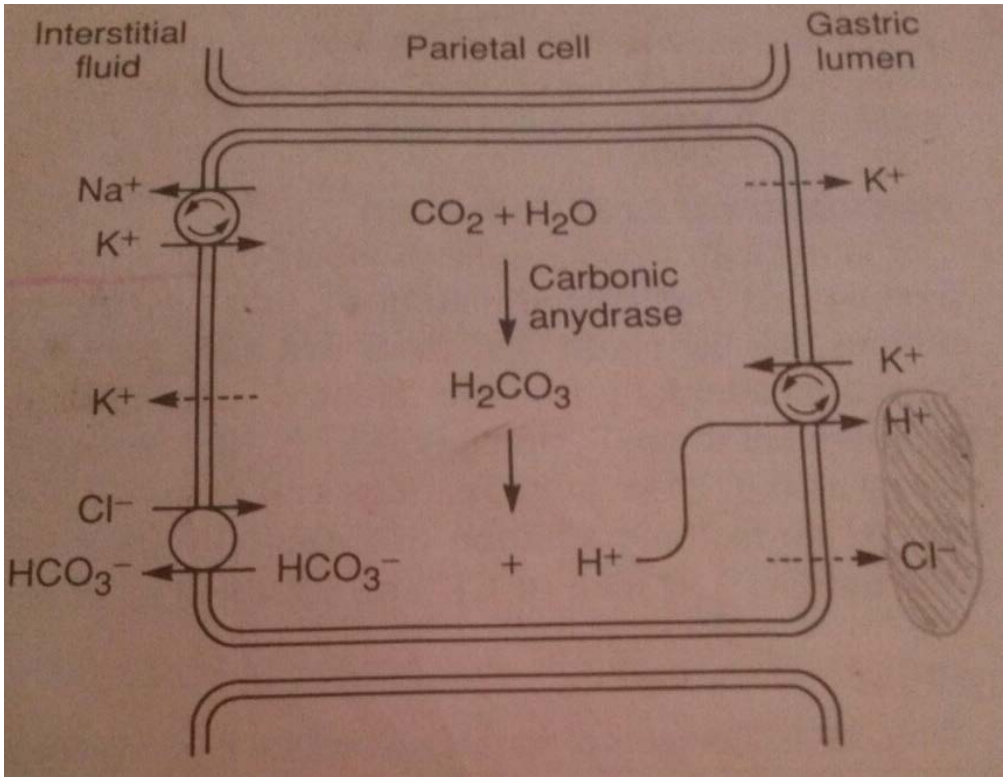

Figure 1c: Formation of gastric acid in parietal cells of the gastric glands (Ganong, 1995)

these diseases. A large number of studies have indicated that most cases of gastric ulcers, gastritis and stomach cancer are caused by *H. pylori* infection. The stomach has to regenerate a new layer of mucosa every two weeks, or else damage to the epithelium may result.

Peptic ulcer

An ulcer may be defined as a deep defect in the esophageal, gastric, duodenal and/ or intestinal wall penetrating the entire mucosal thickness and the muscularis mucosa (Tarnawski, 2000). A peptic ulcer, also known as peptic ulcer disease or *ulcus pepticum*, 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. Peptic ulcer may be found in the stomach or the first part of the duodenum. A gastric ulcer is an ulcer that is located within the stomach only. As many as 70-90% of peptic ulcers are associated with *H. pylori*, a spiral-shaped bacterium that lives in the acidic environment of the stomach. Ulcers can also be caused by drugs such as aspirin and other Non Steroidal Anti Inflammatory Drugs (NSAIDs).

History of peptic ulcer

John Lykoudis, (a general practitioner in Greece) was the first person who treated patients with peptic ulcer disease with antibiotics, long before it was commonly recognized that bacterial infection were a dominant cause for the disease (Marshall, 2002).

H. pylori was discovered in 1982 by two Australian scientists, Robin Warren and Barry J. Marshall as a causative factor for ulcers (Marshall, 1983). In their publication, Warren and Marshall postulated that most stomach ulcers and gastritis were caused by infection with this

bacterium. This was contrary to the earlier belief that ulcers were caused by stress, chewing of gum (Toohey, 1974), or eating spicy food (Marshall and Warren, 1984).

The *H. pylori* hypothesis was poorly received, so in an act of self-experimentation Marshall drank a Petri dish containing a culture of organisms extracted from a patient. He soon developed gastritis thus proving the relationship between the bacterium and the disease (Van Der Weyden *et al*., 2005).

Subsequently, the Center for Disease Control and Prevention, with other government agencies, academic institutions, etc, launched a United States national education campaign to inform health care providers and consumers about the link between *H. pylori* and ulcers (CDC 1974).

Classification of peptic ulcer

Peptic ulcers are classified according to site where they are found: Stomach (called gastric ulcer), Duodenum (called duodenal ulcer), Esophagus (called Esophageal ulcer), and Meckel's Diverticulum (called Meckel's Diverticulum ulcer).

Peptic ulcers are further classified into different types such as: *Type I*: the ulcer is along the lesser curve of the stomach. *Type II*: here two ulcers are present - one gastric, one duodenal. *Type III*: Prepyloric ulcer. The ulcer is found close to the pyloric area. *Type IV*: Proximal gastroesophageal ulcer. The ulcer is found in the upper part of the stomach close to the oesophagus. *Type V*: this type of ulcer is found at any other location in the stomach.

Signs and symptoms of peptic ulcers

Symptoms of a peptic ulcer include the following: abdominal pain, bloating, abdominal fullness, waterbrash (rush of saliva after an episode of regurgitation to dilute the acid in esophagus); nausea, vomiting, loss of appetite, weight loss, hematemesis (vomiting of blood) and melena (tarry, foul-smelling feces due to oxidized iron from hemoglobin). Occasionally, an ulcer can lead to a gastric or duodenal perforation, which leads to acute peritonitis.

Complications of peptic ulcer

Complications of peptic ulcers include bleeding, perforation, Penetration, cancer and scarring. 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 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. Posterior wall perforation leads to pancreatitis. Penetration is when the ulcer continues into adjacent organs such as the liver and pancreas. Scarring and swelling due to ulcers causes narrowing in the duodenum and gastric outlet obstruction. Patient often presents with severe vomiting. Gastric ulcers usually predispose to cancer especially in the presence of *H. pylori* (Cullen *et al.*, 1997).

Causes of peptic ulcer

A major causative factor is chronic inflammation due to *H. pylori* that colonizes the gastric mucosa. The immune system is unable to clear the infection, despite the appearance of antibodies. Thus, the bacterium can cause a chronic active gastritis, resulting in a defect in the

regulation of gastrin production by that part of the stomach. Gastrin secretion may be increased. Gastrin stimulates the production of gastric acid by parietal cells and, in *H. pylori* colonization, the increased gastrin secretion leads to increase in acid output contributing to the erosion of the mucosa and therefore ulcer formation.

Another major cause is the use of NSAIDs. The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins especially PGE_2 and PGI_2. NSAIDs block the function of cyclooxygenase 1 (*COX-1*), which is essential for the production of these prostaglandins. Without the protective mucus, the stomach is susceptible to ulcerstions (Johannessen, 2000).

Although some studies have found correlations between smoking and ulcer formation (Kato *et al,* 1992) others have been more specific in exploring the risks involved and have found that smoking by itself may not be much of a risk factor unless associated with *H. pylori* infection (Kurata *et al.*, 1997; Salih, 2007; Martin *et al.*, 2008). Similarly, while studies have found that alcohol consumption increases risk when associated with *H. pylori* infection, it does not seem to independently increase risk, and even when coupled with *H. pylori* infection, the increase is modest in comparison to the primary risk factor (Salih *et al.*, 2007; Sonnenberg *et al.*, 1981).

Gastrinomas, are rare, gastrin-secreting tumors. They cause multiple and difficult-to- heal ulcers.

Stress also contributes to ulcer formation. *H. pylori* thrives in an acidic environment, and stress has been demonstrated to cause the production of excess gastric acid. This was supported by a study on mice showing that both long-term water-immersion-restraint stress and *H. pylori* infection were independently associated with the development of peptic ulcers (Kim *et al* 2002).

A study of peptic ulcer patients in Thailand also showed that chronic stress was strongly associated with an increased risk of peptic ulcer (Wachirawat *et al.*, 2003).

Diagnosis of peptic ulcer

The diagnosis of peptic ulcer is established based on the characteristic symptoms. Stomach pain is usually the first to signal a peptic ulcer. Confirming the diagnosis is made with the help of tests such as endoscopies or barium contrast x-rays. An esophagogastroduodenoscopy, a form of endoscopy, is carried out on patients in whom a peptic ulcer is suspected. By direct visual identification, the location and severity of an ulcer can be described. Blood tests are not very reliable in diagnosing ulcers. One of the reasons is their inability to differentiate between past exposure to the bacteria and current infection. Additionally, a false-negative is possible with a blood test if the patient has recently been taking certain drugs, such as antibiotics.

The diagnosis of *Helicobacter pylori* can be made by: Urea breath test, direct culture from an gastroscopy biopsy specimen, direct detection of urease activity in a biopsy specimen by rapid urease test, measurement of antibody levels in blood, stool antigen test and histological examination

Macroscopic and microscopic appearance of peptic ulcers

Gastric ulcers are most often localized on the lesser curvature of the stomach. The ulcer is a round or oval defect ("hole"), 2 to 4 cm diameter, with a smooth base and perpendicular borders. These borders are not elevated or irregular in the acute form of peptic ulcer. In the ulcerative form of gastric cancer the borders are irregular. Surrounding mucosa may present radial folds, as a consequence of the parietal scarring.

Microscopically, the ulcer margins appear perpendicular. During the active phase, the base of the ulcer shows 4 zones: inflammatory exudate, fibrinoid necrosis, granulation tissue and fibrous tissue. The fibrous base of the ulcer may contain vessels with thickened wall or with thrombosis.

Treatment of peptic ulcer

Younger patients with ulcer-like symptoms are often treated with antacids or H2 antagonists before endoscopy is done. Bismuth compounds may actually reduce or even clear bacteria.

Patients who are taking NSAIDs may also be prescribed a prostaglandin analogue (Misoprostol) in order to help prevent peptic ulcers, which may be a side-effect of the NSAIDs.

When *H. pylori* infection is present, the most effective treatments are combinations of 2 antibiotics (e.g. Clarithromycin, Amoxicillin, Tetracycline, Metronidazole) and 1 proton pump inhibitor (PPI). In complicated, treatment-resistant cases, 3 antibiotics (e.g. amoxicillin + Clarithromycin + metronidazole) may be used together with a PPI and sometimes with bismuth compound. Treatment of *H. pylori* usually leads to clearing of infection, relief of symptoms and eventual healing of ulcers. Perforated peptic ulcer requires surgical repair of the perforation. Most bleeding ulcers require endoscopy to stop bleeding with cautery, injection, or clipping.

Epidemiology of peptic ulcer disease

The lifetime risk for developing a peptic ulcer is approximately 10% (Snowden, 2008). In the developed countries the prevalence of *H. pylori* infections roughly matches age (i.e., 20% at age 20, 30% at age 30, 80% at age 80 etc.). Prevalence is higher in developing countries. Transmission is by food, contaminated groundwater, and through human saliva. A minor percentage of cases of Helicobacter infection will eventually lead to an ulcer. Previously, peptic

ulcer disease had a tremendous effect on morbidity and mortality until the last 30 years, when epidemiological trends started to point to an impressive reduction in its incidence. The reason why the rates of peptic ulcer disease decreased is due to the development of newer and effective medication, acid suppressants and the discovery of *H. pylori.*

Gastric ulcer has been reported in Nigeria (Ameh and Nmadi, 1998; Onyekwere *et al.*, 2008). However, the National prevalence rate for ulcer in Nigeria is not easily available. In Jos, north-central Nigeria, ulcer prevalence rate is put at 4.9% (Malu *et al*, 1994). In other countries, the annual incidence is:1 case per 1000 population in Japan, 1.5 cases per 1000 population in Norway and 2.7 cases per 1000 population in Scotland (Sanjeeb and Daryl, 2006).

2.3 Pathophysiology of wound healing and inflammation

Ulcer healing is a genetically programmed repair process, includes inflammation, cell proliferation, re-epithelialization, formation of granulation tissue, angiogenesis, interactions between various cells, matrix and tissue remodeling, all resulting in scar formation. Healing of a wound is a complex but generally orderly process of tissue repair and remodeling subsequent to injury. The wound healing response is aimed at reconstituting a tissue closely similar to the original one and can be divided into several different but overlapping phases. The processes involve cellular activation, division, migration and differentiation of many different cell types. Wound healing may ultimately be reduced to a sequence of processes-inflammation, granulation tissue formation, wound contraction collagen accumulation and wound remodeling (Clark,1991). Dysfunctions of the different physiological phases of wound healing may be responsible for disorders of normal wound healing (Clark and Henson, 1996).

In order to balance degradative and regenerative processes these events require a finely tuned control of various biochemical, cellular, and immunological reaction cascades. They are mediated by locally released growth factors and cytokines which may act in autocrine or Paracrine manner. All phases of wound healing are either directly or indirectly controlled by cytokines. It appears that it is the balance of these cytokines and other mediators rather than the mere presence or absence of one or more cytokines that plays a decisive role in regulating the initiation, progression and resolution of wounds. In addition, cell-cell and cell-matrix interactions, mediated, for example by various cell surface adhesion molecules, play an important role in wound healing. Specific cytokines include endothelial growth factor, platelet derived growth factor, tissue necrotic factor, transforming growth factor-beta, vascular endothelial growth factor, Endothelins, angiopoetins, interleukin-1, etc (Mitchell and Cotran, 2003).

Many different cytokines have been shown to be present in wound fluid although their detection does not necessarily correlate with biologic activity. Moreover, individual cytokines can influence wound repair in different ways as they may have diverse effects in similar physiological situations and usually have more than one specific effect on cells. Their effects may be different in different cell populations, depending, among other things, on the presence or absence of other factors, the concentration of bioactive factors, and on the repertoire of suitable receptors expressed by the cells in question (Mitchell and Cotran, 2003).

The first step in the wound healing process involves the activation of the intrinsic part of the blood coagulation cascade. This event is triggered by the rupture of blood vessels and initiated

by the contact of plasma with tissue and basal membranes of cells and the exposure of subendothelial collagen to platelets (Yamaguchi and Yoshikawa, 2001).

Inadequate clot formation is associated with impaired wound healing. The locally formed fibrin clot effectively serves as a scaffolding matrix that can be colonized subsequently by inflammatory cells such as neutrophils, monocytes and macrophages and fibroblasts in that sequence. The phases of normal wound healing follow an orderly sequence of events that are characterized by, and regulated by, the chronologic appearance of a number of different cell types (Mitchell and Cotran, 2003).

Once migration of cells into the wound milieu has been achieved this is followed by cell activation. Cell activation is the phenotypic alterations of cellular, biochemical, and functional properties of a cell. Cell activation is initiated by the release of factors from aggregated thrombocytes. It has fundamental implications in several aspects of wound healing. This process leads, among other things, to the expression of new cell surface antigens, increased cytotoxicity, and increased production and release of cytokines.

Fibroblasts deposit the collagen that forms part of the substance of granulation tissue formed later during wound healing. Granulation tissue develops from the connective tissue surrounding the damaged or missing area and contains mainly small vessels, inflammatory cells fibroblasts etc. The formation of new blood vessels (angiogenesis) is initiated by endothelial cells migrating and proliferating into the healing wound. Angiogenesis is essential for the normal function of fibroblasts and leukocytes.

The final phase of wound healing is characterized by the gradual replacement of granulation tissues by connective tissue and tissue remodelling. This process also requires locally acting

cytokines, metalloproteinases, collagenases and serine proteinases. However, little is known about the factors and mechanisms that eventually restrain tissue growth once the repair process has been completed (Singer and Clark, 1999).

Closing of the wound and the evolution of a scar is associated with a striking decrease in cellularity, including disappearance of typical myofibroblasts. Programmed cell death (apoptosis) probably is the mechanism responsible for the evolution of granulation tissue into a scar.

Factors that affect wound healing

Several factors can affect healing of wounds. Some of them are: malnutrition, blood flow and oxygen delivery, impaired inflammatory and immune response, infection and foreign bodies, age and drugs. Inhibition of prostaglandin synthesis by drugs such as indomethacin can exert injurious actions on the gastric and duodenal mucosa as it abrogates several prostaglandin-dependent defence mechanisms. Inhibition of COX-2 leads to increased gastric acid secretion, decreased mucus and bicarbonate secretion, reduced mucosal blood flow and vascular injury, leucocyte accumulation, and reduced cell turnover. All these factors will contribute to the genesis of mucosal damage (Rainsford and Rainsford, 1997). Within this broad spectrum of events, the microvascular damage appears to play a central role. Prostaglandins of the E and I series are potent vasodilators that are continuously produced by the vascular endothelium. Inhibition of their synthesis by an NSAID leads to vasoconstriction (Gana *et al.,* 1987). Furthermore, inhibition of prostaglandin formation results in a rapid and significant increase in the number of neutrophils adhering to the vascular endothelium in both gastric and mesenteric venules (Wallace *et al.,* 1993). Neutrophil adherence in turn causes microvascular stasis and

mucosal injury through ischaemia and release of oxygen derived free radicals and proteases (Vaananen *et al.*, 1991).

Some of the most commonly encountered impediments to wound healing include tissue hypoxia, infection, presence of debris and necrotic tissue, use of anti-inflammatory medications, a diet deficient in vitamins or minerals, or general nutritional deficiencies, tumors, environmental factors, and metabolic disorders such as diabetes mellitus. An intact microcirculation is required for tissue nutrition, removal of waste products, inflammatory responses and temperature regulation therefore, logically any defect in microvascular function adversely affects tissue repair (Stadelmann *et al.*, 1998). Wound healing occurs faster in younger individuals than older ones. This is because younger people have greater capacity for repair. Their cells respond faster to cytokines and pass through the healing process more efficiently than the tissues of an elderly individual (Garvin, 1990).

Protein is essential for the synthesis of collagen structures that establish wound tensile strength. Without adequate tensile strength of collagen structures, dehiscence and evisceration can occur. Protein deficiencies also impair formation of new capillaries, fibroblastic activity, etc. When fatty acids, which are used to form cell membranes, are used for energy, as in poorly controlled diabetes mellitus, healing is impaired, since there is delayed cell membrane synthesis. Decreased chemotaxis, phagocytosis, bacterial killing (Marhoffer, et al, 1992; Kwoun *et al.*, 1997), decrease antioxidant synthesis, and increased oxygen free radical generation (Mohan and Das, 1998) during the early phase of wound-healing all have been implicated in impaired healing (Moulin *et al.*, 1998).

In conclusion, healing of ulcer is accomplished by filling the mucosal defect with cells migrating from the ulcer margin and by connective tissue, including microvessels originating from granulation tissue. Speed and quality of ulcer healing depend, among other factors, on (1) epithelial cell migration and proliferation in the mucosal ulcer margin, (2) angiogenesis in the ulcer bed, (3) maturation and contraction of the granulation tissue in the ulcer bed, and (4) quality of remodelling of epithelial and mesenchymal structures in the late healing phase (Schmassmann, 1998).

2.4 Lead

Lead (Pb) is a dense, bluish-gray metallic element. It is one of the first known metals. It is malleable, ductile, and a poor conductor of heat and electricity. It has atomic number of 82, and atomic weight of 207. Lead exists in four different isotopes. The element is in group 14 (o r IVa) of the periodic table. It is highly durable and resistant to corrosion. Its low melting point of 327 °C coupled with its easy castability, softness and malleability, make lead and lead alloys

especially suitable for a wide range of cast products, including battery grids and terminals, counterweights, plumbing components, and type metal. With a specific gravity of about 11.35

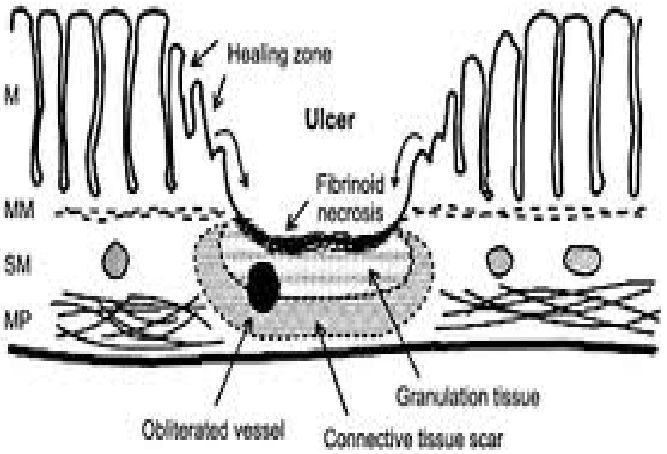

Figure 2: Diagrammatic representation of ulcer healing. M, mucosa; MM, muscularis mucosa; SM, submucosa; MP muscularis propria. Copyright: journals.prous.com/google images

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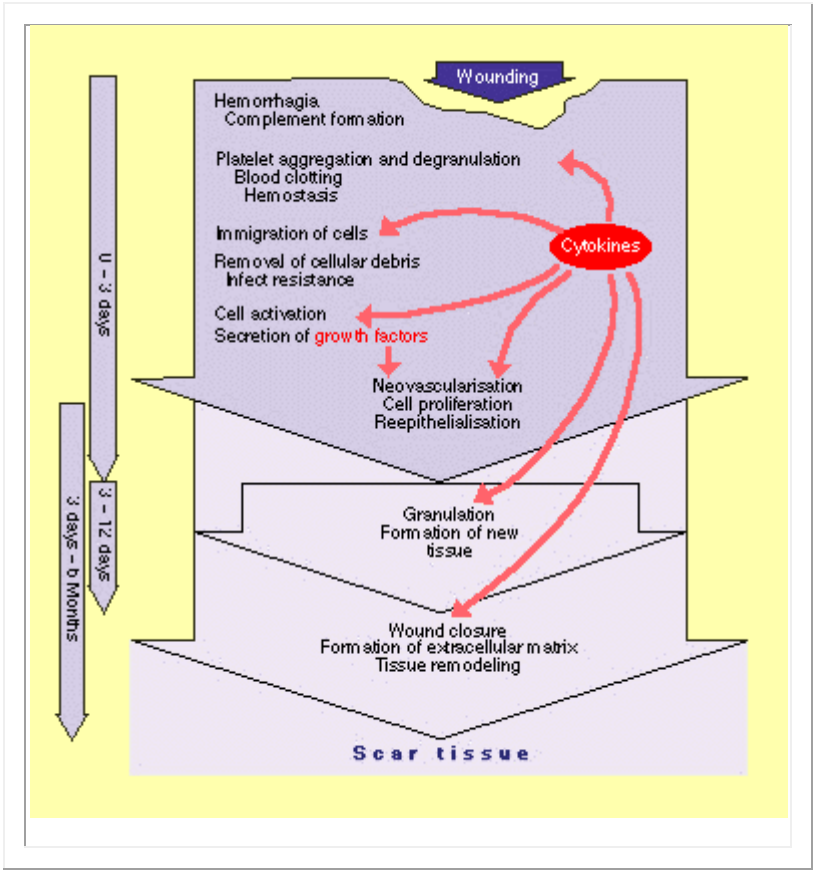


Figure 3: Schematic representation of processes involved in wound healing copyright: copewithcytokines.de/google images

grams per cubic centimeter, lead is the densest of the common metals, except for gold; this makes it a good shield against X- rays and gamma radiation. Its combination of density and softness make it an excellent barrier to sound. It has excellent corrosion resistance when it can form an insoluble protective coating on its surface. The metal has a face-centered cubic crystal lattice structure. It has low tensile strength and is a poor conductor of electricity. A freshly cut surface has a bright silvery luster, which quickly turns to the dull, bluish-gray color characteristic of the metal. Lead melts at 327 °C and boils at 1749 °C. Lead has four stable isotopes, all of which are the end products of the radioactive decay of other elements; their relative abundances are: lead-204, 1.48 percent; lead-206, 23.6 percent; lead-207, 22.6 percent; and lead-208, 52.3 percent. Lead is soluble in nitric acid but is little affected by sulfuric or hydrochloric acids at room temperature. In the presence of air, it slowly reacts with water to form lead hydroxide, which is slightly soluble. Lead is toxic when ingested so pipes used for carrying drinking water should not contain lead (Polyanskiy, 1986).

About 30 percent of all lead consumed is in the form of lead compounds, such as oxides, tetraethyl and tetramethyl lead, lead chromates, sulfates, silicates, carbonates, and organic compounds. These lead compounds have been used in paste mixtures in storage batteries, in cements, glasses, and ceramics, as pigments in paints, and as an antiknock agent in gasoline (Lide, 2004)

Lead is widely distributed all over the world in the form of its sulfide, the ore galena. Lead ranks about 36th in natural abundance among elements in Earth's crust. Ores of lead include cerussite, galena and anglesite. The principal method of extracting lead from galena is to roast the ore, that is, convert it to the oxide, and reduce the oxide with coke in a blast furnace. Another method is to roast the ore in a reverberatory furnace until part of the lead sulfide is converted to lead oxide

and lead sulfate. The air supply to the furnace is then cut off and the temperature raised; then the original lead sulfide combines with the lead sulfate and lead oxide to form metallic lead and sulfur dioxide (Lide, 2004).

Waste materials, such as battery scrap, recovered from various industrial processes, is also smelted and constitutes an important source of lead. Pig lead is often purified by stirring molten

lead in the presence of air. The oxides of the metallic impurities rise to the top and are skimmed off. The purest grades of lead are refined electrolytically (Lide, 2004).

History of lead use

Lead was mentioned in the Old Testament of the Bible. It was used by the Romans for making water pipes, soldered with an alloy of lead and tin. Lead has been mined and smelted for at least 8,000 years. This is confirmed by artifacts in various museums and by ancient histories and other writings, including the biblical Book of Exodus. Lead beads found in what is now Turkey have been dated to around 6500 BC, and the Egyptians are reported to have used lead along with gold, silver, and copper as early as 5000 BC. In Pharaonic Egypt, lead was used to glaze pottery and make solder as well as for casting into ornamental objects. The British Museum holds a lead figure, found in the temple of Osiris in the ancient city of Abydos in western Anatolia that dates from 3500 BC (Heskel, 1983).

One of the most important historical applications of lead was the water pipes of Rome. Lead pipes were fabricated in 3-metre (10-foot) lengths and in as many as 15 standard diameters. Many of these pipes, still in excellent condition, have been uncovered in modern-day Rome and

England. The Roman word plumbum, denoting lead water spouts and connectors, is the origin of the English word plumbing (Rooney, 2007).

Marcus Vitruvius Pollio, a 1st-century-BC Roman architect and engineer, warned against the use of lead pipe for conveying water, recommending that clay pipes be used instead. Others believed lead to have favorable medical qualities. Pliny, a Roman scholar of the 1st century AD, wrote that lead could be used for the removal of scars, as a liniment, or as an ingredient in plasters for ulcers and the eyes, among other health applications (Stellman, 1998).

In 1859 a French physicist, Gaston Planté, discovered that pairs of lead oxide and lead metal electrodes, when immersed in a sulfuric acid electrolyte, generated electrical energy and could subsequently be recharged. A series of further improvements by other scientists led to commercial production of lead-acid storage batteries 40 years later. The huge growth of battery markets in the 20th century (eventually consuming about 75 percent of the world's lead production) largely followed the rise of the automobile, in which batteries were used for powering virtually all parts of the vehicles. Another major lead product was tetraethyl lead, a gasoline additive invented in 1921 in the United States. It solved the problem of “knocking” in vehicle engines that operate at high temperatures. Soon after reaching its peak 50 years later, the use of this lead compound declined in the United States. The installation of catalytic converters became mandatory on the exhaust systems of all American passenger cars (Crompton, 2000).

Ores of lead

There are more than 60 known lead-containing minerals, but the most important primary ore of the metal is the lead sulfide galena (PbS). Galena often contains silver, zinc, copper, cadmium, bismuth, arsenic, and antimony. Other commercially significant lead-containing minerals are

cerussite (lead carbonate) and anglesite (lead sulfate). These are known as secondary minerals in that they derive from galena through natural actions, such as weathering. Cerussite, for instance, is formed by the action of carbonate groundwater on galena, whereas anglesite is formed when galena is subjected to sulfate solutions generated from the oxidation of sulfide minerals (Sutherland *et al.,* 2005).

More than 95 percent of mined lead comes from these three ores. Ores of commercial importance may range from 2 to 20% lead or more, even though galena itself contains 87% lead. The percentage of recoverable lead in ores is typically about 4%. More than 50% of the total lead refinery demand is met by the recycling of spent lead. Large deposits of lead ores are located in Australia, Canada, China, Mexico, Peru, Kazakhstan, Russia and the United States. (Hong *et al.,* 1994)

Mining and concentrating of lead

Once the ore is obtained from the mines, usually at depths of about 60 meters, it is treated at concentrating mills. Here the ore is finely crushed and then treated by one of several mineral concentration processes. (Sutherland *et al*, 2005).

Flotation separation is generally used for sulfide ores. In this process, the crushed ore is diluted with water and agitated violently with air in a tank to which chemicals have been added. The sulfide particles attach themselves to the chemicals. Air is then bubbled into the mixture. This causes an oily froth containing most of the lead of the ore to float to the top. Aggregation of the metallic concentrate is initiated in the flotation bath. The froth obtained here then flows from the

tank and is dried. At this stage, lead concentrates leaving the concentration mill to the smelter plant contain 40 to 80% lead, with varying amounts of impurities e.g. sulfur, zinc etc.

Extraction of lead

The lead concentrate is roasted to remove sulphur and further agglomerate it. Various fluxing materials, such as limestone and iron ore are mixed with the ore concentrate. The mixture is spread on a moving grate, and air is blown through at a temperature of 1,400°C. The sulfur, along with coke, serves as a fuel and is combusted to sulfur dioxide gas (Sutherland, 2005).

Roasting fuses the remaining ingredients into a brittle product called sinter, which consists of oxides of lead, zinc, iron, and silicon along with lime, metallic lead, and some remaining sulfur. This material is then broken into lumps. The prefluxed, lumpy sinter is then loaded into the top of a heated blast furnace, along with the coke fuel. A blast of air is admitted to the lower part of the furnace to aid combustion of the coke, generating a temperature of about 1,200 °C and producing carbon monoxide. This gas then reacts with the metallic oxides, producing carbon dioxide and molten metal (Sutherland, 2005).

When reduction is complete, the lead is drawn off to flow into drossing kettles or molds. At this stage, the semi-finished product contains 95 to 99% lead and other dissolved metallic and nonmetallic impurities is known as base bullion. The bullion is maintained at a temperature just above its melting point, about 330 °C. At this temperature copper and other impurities are easily removed. At the end of the process, the remaining bullion is now refined to yield lead of commercial quality (Sutherland, 2005). Later, more advanced refining processes have been developed such as direct smelting processes conducted in relatively small, intensive reactors.

Direct smelting can be divided into two categories: (1) submerged smelting, as in the QSL and Isasmelt processes and (2) suspension smelting, as in the KIVCET process. KIVCET is a Russian acronym for “flash-cyclone-oxygen-electric-smelting”(Sutherland, 2005).

Refining of Lead

To remove and recover remaining impurities from lead bullion, either pyrometallurgical or electrolytic refining is used.

The Parkes zinc-desilvering process is the most widely used pyrometallurgical method of refining lead bullion. As in smelting, the lead is first melted and again allowed to cool below the freezing point of copper, which crystallizes and, along with any remaining nickel, cobalt, and zinc, is removed by skimming. The lead mix then passes to a reverberatory “softening” furnace, where the temperature is raised and the molten lead is stirred. A blast of air oxidizes any remaining antimony or arsenic (Sutherland, 2005).

After softening, the lead goes to desilvering kettles, where small quantities (less than 1 percent by weight) of zinc are added. With stirring, the molten zinc reacts to form compounds with gold and silver, both of which are more soluble in zinc than in lead. The compounds are lighter than the lead, so that, on cooling to below 370 °C but above the melting point of lead, they form a crust that is removed and taken to a parting plant for recovery of the precious metals. The remaining zinc is then removed by reheating the molten lead to 500 °C and creating a vacuum over the surface.

The Harris process of softening and dezincing is designed to remove impurities from desilvered lead by stirring a mixture of molten caustic salts at a temperature of 450–500 °C into the molten

lead. Metallic impurities react with the chemicals and are collected in the form of their oxides or oxysalts.

Lead bullion containing more than 0.1 percent bismuth can be purified by the Betterton-Kroll process, which usually follows softening, desilvering, and dezincing and involves treatment of the melt with calcium and magnesium. Bismuth unites with these metals to form compounds that

rise to the surface. The compounds are skimmed off and treated for recovery of bismuth, a valuable by-product (Sutherland, 2005).

The Betterton-Kroll process produces a refined lead with bismuth contents of 0.005 to 0.01 percent. When a refined lead of higher purity is required, or when a lead bullion high in bismuth has to be refined, employment is made of electrolytic refining. This process is costly, but it has the major advantage of separating lead from every impurity except tin in one vessel or one stage, and it does so without emitting lead-bearing fumes or gases. The bullion is cast into large plates, which are hung as anodes in electrolytic tanks where they dissolve. Pure lead is deposited on a thin sheet of lead that serves as the cathode.

Secondary lead is lead derived from scrap. Accounting for nearly half of the total output of refined lead, it is a significant factor in the lead market because it is easily melted and refined and rarely becomes contaminated by impurities during service. About 85 percent of secondary lead comes from discarded automobile batteries. The imposition of stringent environmental regulations governing disposal of spent batteries has led to greater recycling efforts that will ensure the growth of this supply.

The recycling of lead from battery scrap involves treating and separating the scrap, reducing and smelting the lead-containing fractions, and refining and alloying the lead bullion into a

commercial product. It is usually conducted in reverberatory and blast furnaces at refineries devoted exclusively to handling secondary lead and lead alloys. However, some primary refineries also refine secondary lead; this has led to a growing use of rotary furnaces, which are batch kilns that are rotated during the smelting process (Sutherland, 2005).

Secondary raw materials are usually processed separately. Sometimes, however, lead residues, sludges, or flue dusts are mixed with oxides from the battery treatment plant and processed together (Sutherland, 2005).

Purity levels of lead

Refined lead usually has a purity of 99 to 99.99 percent, but lead of 99.999 percent purity is becoming common commercially. At these levels, the grades of lead differ mostly by their bismuth content. With modern smelting and refining techniques, it is possible to reach these high levels of purity regardless of the nature of the raw material. Grades of very high purity have been produced, largely for scientific and research purposes (Crompton, 2000).

Because the mechanical properties of pure lead are relatively poor, it is alloyed with other elements, particularly to improve strength or hardness. Lead and most of its many alloys may be readily fabricated by almost all commercial processes. Extruded products include pipe, rod, wire, ribbon, traps, and special shapes. Rolled products, which may range in thickness from foil some 10 micrometers (0.0004 inch) thick to sheets 5 centimeters (2 inches) thick or more, are used in many applications. These include corrosion-resistant equipment (particularly for handling sulfuric acid) for the chemical industry; roofing, flashing, waterproof membranes, and similar applications; in X-ray and gamma-ray shielding and in sound isolation, sometimes as a laminate

in a plastic sandwich; and as vibration-damping pads or housings for many building and machinery applications.

Alloys of lead

(a) Antimonial lead

The most common and important metal alloyed with lead is antimony. Antimonial lead alloys usually contain from 1 to 6 percent antimony, but they may contain as much as 25 percent. Other components usually include tin, iron, copper, zinc, silver, arsenic, or traces of nickel. Because it has improved hardness and strength, Antimonial lead has traditionally been known simply as hard lead.

Antimonial lead loses strength rapidly at elevated temperatures, so that it is generally used in applications where temperatures do not exceed 120 °C. By far its most important commercial application is as the cast metal for grids and terminals in lead-acid storage batteries, in which the antimony content ranges up to 8 percent. “Maintenance-free” automotive batteries are usually produced with 1.5 to 3 percent Antimonial-lead negative plates and positive plates containing 0.04 to 0.06 percent calcium. Other important lead-antimony applications include pipe and sheet, cable sheathing, and ammunition.

(b) Lead-based bearing alloys

Lead-based bearing alloys, also known as lead-based babbitt metals or white metals, are usually Antimonial lead with widely variable additions of tin or copper (or both) and arsenic to increase strength. One such alloy, commonly used for railroad-car journal bearings, contains 86 percent lead, 9 percent antimony, and 5 percent tin. Many alloys of lead and alkaline-earth metals, such

as calcium and sodium, also are widely used as bearing materials. Leaded bronzes contain from 4 to 25 percent lead plus additions of copper and tin, and some copper-lead bearing alloys contain up to 40 percent lead. All these bearing alloys are sufficiently soft so that lubrication failure does not result in damage to the bearing.

(c) Lead-Tin alloys

Lead-tin alloys containing up to 98 percent by weight tin are used as solders. The strengths of these alloys increase with higher tin content, while the melting point is lowered to a minimum of 183 °C with a lead content of 38 percent. A half-lead–half-tin alloy is the most common general-purpose solder. Considerably lower tin contents, from around 5 to 30 percent, are used by the automotive industry for soldering radiator cores and for other applications. Tin contents as low as 2 percent are used in the canning industry. The electronics industry requires low-melting solders to protect heat-sensitive components, and so tin contents generally are around 60 to 65 percent. Terne metal, an alloy of lead and typically 10 to 15 percent tin, is used to coat steel sheet in order to produce a strong, corrosion-resistant product that is widely used for automobile gasoline tanks, packaging, roofing, and other uses where lead's favorable properties are sought but a reduced total weight is important.

(d) Lead-silver

When solder joints are desired that retain their strength and other properties at higher temperatures than conventional lead-tin solders, use is made of lead-silver alloys that have melting points of about 305 °C. The silver content of these soldering alloys ranges from 1.5 to 1.75 percent; tin is commonly added at a level of about 1 percent to inhibit intergranular

corrosion. Adding 1% silver to lead-antimony alloys enhance their performance as a grid material in car batteries.

Chemical compounds of lead

There are many organic and inorganic lead compounds, including oxides, carbonates, sulfates, chromates, silicates, and acetates. Most of these are manufactured from high quality corroding lead.

Oxides

Lead monoxide, or litharge (PbO), a yellow, crystalline powder formed by heating lead in air. It is used in making flint glass, as a drier in oils and varnishes, and in the manufacture of insecticides. Red lead, or minium (Pb_3O_4), a scarlet, crystalline powder formed by oxidizing lead monoxide, is the pigment in paint used as a protective coating for structural ironwork and steelwork Litharge, or lead monoxide (PbO), is one of the most important of all metal compounds. It is manufactured by the oxidation of metallic lead in a variety of processes, each resulting in a distinctive variation in physical properties. Hence, it is available in many particle sizes and in two crystal forms.

The largest single use of litharge is as a paste material for storage batteries. Very high purity litharge is widely used in the production of glass, including television picture tubes and computer video display terminals, where lead's shielding powers block otherwise harmful radiation. In addition, the inner portion of the common light bulb is made of leaded glass. Litharge is employed in the manufacture of enamels and in the production of frits, which are fluxes used to reduce melting temperatures in glass production. The lead in litharge imparts greater strength and brilliance to fine crystal glassware.

Red lead, or lead tetroxide (Pb_3O_4), is another lead oxide whose two most important uses are in paints and as an addition to litharge in storage batteries. It also has significant application in glasses, glazes, and vitreous enamels. Red lead is produced by heating litharge at a carefully controlled temperature, lower than that used for the manufacture of litharge. In this process, the litharge takes on more oxygen to form red lead.

Lead dioxide (PbO_2) is an oxidizing agent used in the manufacture of dyes, matches, and rubber substitutes. Orange mineral (Pb_3O_4), having the same chemical composition as red lead but differing in colour and tone, is exploited in the manufacture of printing inks and pigments. Black oxide is a mixture of litharge and finely divided metallic lead and is primarily used in batteries.

Lead (IV) oxide, found naturally as the brown mineral plattnerite, is commercially produced from trilead tetroxide by oxidation with chlorine. Lead (IV) oxide is used as an oxidizing agent in the production of dyestuffs, chemicals, pyrotechnics, and matches and as a curing agent for polysulfide rubbers. Trilead tetroxide (known as red lead or minium) is produced by further oxidation of lead (II) oxide. It is the orange-red to brick-red pigment commonly used in corrosion-resistant paints for exposed iron and steel. It also reacts with iron (III) oxide to form a ferrite used in making permanent magnets (Polyanskiy 1986).

Tetraethyl lead

Tetraethyl lead ($Pb[C_2H_5]_4$), usually designated TEL, was once a major industrial compound of lead because of its use as an antiknock agent in gasoline. But its use has declined since the mid-1970s owing to environmental restrictions. TEL is produced by melting pig lead and mixing it with sodium to form a lead-sodium alloy that is solidified and ground. This alloy is reacted with ethyl chloride to form TEL. It is considered a significant contributor to air pollution.

Silicates

Lead silicates are considered to be the most versatile of lead chemical compounds in the ceramics industry. Three types are available commercially: lead monosilicate ($PbO \cdot O.67SiO_2$), lead bisilicate ($PbO \cdot 0.03A1_2O_3 \cdot 1.95SiO_2$), and tribasic lead silicate ($PbO \cdot O.33SiO_2$). The silicates are used extensively in finely divided form for glazes or certain ceramic bodies and in granular form for glass, dry-process enamels, or in frit batches. The lead silicates also yield white pigments for exterior paints.

Lead acetate

Another significant compound is lead (II) acetate, $Pb(C_2H_3O_2)_2$, a water-soluble salt made by dissolving litharge in strong acetic acid. The common form, the trihydrate [$Pb(C_2H_3O_2)_2 \cdot 3H_2O$], called sugar of lead, is used as a mordant in dyeing and as a drier in paints. In addition, it is utilized in the production of other lead compounds. This was the salt used for this study.

Carbonates

The first white paint pigment was white lead carbonate [$2PbCO_3 \cdot Pb(OH)_2$]. Today it is produced by several different methods, including chemical precipitation. Although it is no longer used as a pigment in paints owing to its role in lead poisoning, white lead is still used, though in decreasing amounts, in ceramics. Its decline in the latter use is due to its solubility in many acid solutions, including gastric juice, with possibly hazardous results. Basic lead carbonate, $(PbCO_3)_2 \cdot Pb(OH)_2$, called white lead, has been used for over 2000 years as a white pigment. It is also used in ceramic glazes and in making other pigments. It may be produced by processes such as electrolysis or forcing of hot air and carbon dioxide through large rotating cylinders containing powdered lead and acetic acid (Nakashima et al., 2007).

Chromates

Lead chromates—yellow, orange, and red—have significant application as pigments. The standard chromate, $PbCrO_4$, is made by precipitation from solutions of lead acetate or lead nitrate to which potassium or sodium bichromate has been added. Gradations of colour are obtained by varying the types of lead chromates used as well as by the process of manufacture. Lead chromate, or chrome yellow ($PbCrO_4$), a crystalline powder used as a yellow pigment, is prepared by the reaction of lead acetate and potassium bichromate.

Other compounds of lead

A whole series of lead compounds are used as stabilizers in vinyl plastics. As such, they enable the plastics to resist degradation from heat and light, especially when used outdoors. These stabilizers include certain basic sulfates and phosphites, complex silicates, and organic compounds such as salicylates and basic stearates.

Among other lead compounds of interest are lead azide [$Pb(N_3)_2$], a standard detonator explosive whose efficiency is less affected by moisture than others and is virtually insoluble in water, and lead arsenate, derived from litharge and used as an insecticide. Various other salts, most notably basic lead carbonate, basic lead sulfate, and basic lead silicate, were once widely employed as pigments for white exterior paints. Since the mid-20th century, however, the use of such so-called white lead pigments has decreased substantially because of a concern over their toxicity and attendant hazard to human health. The use of lead arsenate in insecticides has virtually been eliminated for the same reason.

Uses of lead

Lead is used in large quantities in storage batteries and in sheathing electric cables. Large quantities are used in industry for lining pipes, tanks, and X-ray apparatus. Because of its high density and nuclear properties, lead is used extensively as protective shielding for radioactive material. Among numerous alloys containing a high percentage of lead are solder, type metal, bearing metals, etc. A significant amount of lead is consumed in the form of its compounds, particularly in paints and pigments.

Because of this general chemical resistance, considerable amounts of lead are used in roofing, as coverings for electric cables placed in the ground or underwater, and as linings for water pipes and conduits and structures for the transportation and processing of corrosive substances (Rooney, 2007).

Lead has many other applications; the largest is in the manufacture of storage batteries. It is used in ammunition and as a constituent of various low-melting alloys, such as solder, type metal, and pewter. In the construction of large buildings, lead sheets are used in the walls to block the transmission of sound; and pads of lead and asbestos are used in the foundations to absorb the vibrations caused by street traffic and other sources. It is also used as a protective shielding around nuclear reactors, particle accelerators, X-ray equipment, and containers used for transporting and storing radioactive materials (Rooney, 2007).

2.5 Lead poisoning and toxicity

Lead poisoning (also known as plumbism, colica pictonum, saturnism**,** etc) is a medical condition caused by increased levels of the heavy metal lead in the body. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, reproductive and nervous systems. It interferes with the development of the

nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders. Symptoms include abdominal pain, headache, anemia, paralysis, weakness, irritability, reduced intelligence, delayed motor development, and impaired memory, hearing problems and troubles in balance and in severe cases seizures, coma, and death (Golub, 2005; Bergeson and Lynn, 2008).

Humans have been mining and using this heavy metal for thousands of years, and in the process, exposing themselves to its toxicity. Although lead poisoning is one of the oldest known occupational and environmental hazards, the modern understanding of the small amount of lead necessary to cause harm did not come about until the latter half of the 20th century. No safe threshold for lead exposure has been discovered—that is, there is no known amount of lead that is too small to cause the body harm. Lead taken internally in any of its forms is highly toxic; the effects are usually felt after it has accumulated in the body over a period of time. Modern treatment of lead poisoning includes the administration of calcium disodium ethylenediaminetetraacidic acid, or EDTA, a chelating agent; lead is removed from the body by displacing the calcium in EDTA and forming a stable complex that is excreted in the urine

Routes of exposure to lead include contaminated air, water, soil, food, and consumer products. Occupational exposure is a common cause of lead poisoning in adults. One of the largest threats to children is lead paint that exists in many homes, especially older ones. Prevention of lead exposure can range from individual efforts to nationwide policies or international agreements/protocols (e.g. laws that ban lead in products or reduce allowable levels in water or soil).

Elevated lead in the body can be detected by the presence of changes in blood cells visible with a microscope and dense lines in the bones of children seen on X-ray. However, the main tool for diagnosis is measurement of the blood lead level. Different treatments are used depending on the level of lead seen in the test. The major treatments are removal of the source of lead and chelation therapy (Golub, 2005; Bergeson and Lynn, 2008).

Classification of lead poisoning

The Classical definition for "lead poisoning" or "lead intoxication" is exposure to high levels of lead typically associated with severe health effects (Grant, 2009). Poisoning is a pattern of symptoms that occur with toxic effects from mid to high levels of exposure; toxicity is a wider spectrum of effects, including subclinical ones (those that do not cause symptoms) (Guidotti and Ragain, 2007). However, professionals often use "lead poisoning" and "lead toxicity" interchangeably, and official sources do not always restrict the use of "lead poisoning" to refer only to symptomatic effects of lead (Guidotti and Ragain, 2007).

The amount of lead in the blood and other tissues, as well as the time course of exposure, determine toxicity (Pearson, 2003) Lead poisoning may be acute (from intense exposure of short duration) or chronic (from repeat low-level exposure over a prolonged period), but the latter is much more common (Trevor, 2007). In this study, chronic lead exposure was employed in bringing about lead poisoning in the experimental animals. Diagnosis and treatment of lead exposure are based on blood lead level (the amount of lead in the blood), measured in micrograms of lead per deciliter of blood (μg/dL). The US Center for Disease Control and Prevention and World Health Organization state that a blood lead level of 10 μg/dL or above is a cause for concern. However, lead may impair development and have harmful health effects even

at lower levels, and there is no known safe exposure level (Rossi, 2008). Authorities such as the American Academy of Pediatrics define lead poisoning as blood lead levels higher than 10 μg/dl (Ragan and Turner, 2009).

Lead forms a variety of compounds and exists in the environment in various forms as has been outlined above (Grant 2009). Features of poisoning differ depending on whether the agent is an organic or inorganic compound. Organic lead poisoning is now very rare, due to the fact that countries across the world have phased out the use of organic lead compounds as petrol additives, but such compounds are still used in industrial settings (Kosnett, 2007). These organic lead compounds which cross the skin and respiratory tract easily, affect the central nervous system mainly (Kosnett, 2007).

Signs and symptoms of lead poisoning

Lead poisoning can cause a variety of signs and symptoms which vary depending on the individual and the duration of lead exposure. Sometimes, symptoms are nonspecific and may be subtle, and someone with elevated lead levels may have no symptoms (Mycyk, 2005). Symptoms usually develop over weeks to months as lead builds up in the body during a chronic exposure, but acute symptoms from brief, intense exposures also occur (Mycyk, 2005). Symptoms from exposure to organic lead, which is probably more toxic than inorganic lead due to its lipid solubility, occur rapidly (Timbrell, 2008). Poisoning by organic lead compounds has symptoms predominantly in the central nervous system, such as insomnia, delirium tremor and convulsions (Kosnett, 2007).

Symptoms may be different in adults and children; the main symptoms in adults are headache, abdominal pain, memory loss, male reproductive problems, weakness, pain, or tingling in the

extremities (Pearce, 2007). The classic signs and symptoms in children are loss of appetite, lethargy, abdominal pain, vomiting, weight loss, constipation, anemia, kidney failure, irritability, and behavior problems (Pearce, 2007). Children may also experience hearing loss, delayed growth, drowsiness, clumsiness, or loss of new abilities, especially speech skills (Mycyk, 2005) Symptoms may appear in children at lower blood lead levels than in adults (Marshall and Bangert, 2008).

Early symptoms of lead poisoning in adults are commonly nonspecific and include depression, loss of appetite, intermittent abdominal pain, nausea, diarrhea, constipation, and muscular pain (Merrill et al., 2007). Other early signs in adults include malaise, fatigue, decreased libido, and sleep problems (Karri *et al.,* 2008). Unusual tastes in the mouth and personality changes are also early signs (Patrick, 2006). In adults, symptoms can occur at levels above 40 μg/dL, but are more likely to occur only above 50–60 μg/dL (Karri *et al.,* 2008). Symptoms begin to appear in children generally at values of 60 μg/dL (Needleman, 2004). However, the lead levels at which symptoms appear vary widely depending on unknown characteristics of each individual (Bellinger, 2004). At blood lead levels between 25 and 60 μg/dL, neuropsychiatric effects such as delayed irritability and difficulty in concentrating, as well as slowed motor nerve conduction and headache can occur. Anemia may appear at blood lead levels higher than 50 μg/dL (Merrill *et al.,* 2007). Signs that occur in adults at blood lead levels exceeding 100 μg/dL include wrist and foot drop, delirium, coma, headaches, etc (Henretig, 2006). In children, signs of encephalopathy such as bizarre behavior, discoordination, and apathy occur at lead levels exceeding 70 μg/dL (Henretig, 2006). For both adults and children, it is rare to be asymptomatic if blood lead levels exceed 100 μg/dL (Kosnett, 2005).

Routes of exposure to lead

Lead is a very common environmental pollutant (Ragan and Turner, 2009). Causes of environmental contamination include industrial use of lead, such as is found in plants that

process lead-acid batteries or produce lead wire or pipes, and metal recycling and foundries. Children living near facilities that process lead, such as smelters, have been found to have unusually high blood lead levels. In August 2009, parents rioted in China after lead poisoning was found in about 200 children living near zinc and manganese smelters (Watts, 2009). In Zamfara state, northwestern Nigeria, about 300 children died from lead poisoning in early 2010 and 4000 others were affected due to illegal gold mining in the area. Lead exposure can occur from contact with lead in air, household dust, soil, water, and commercial products (Rossi, 2008).

(a) Occupational exposure

In adults, occupational exposure is the main cause of lead poisoning. People can be exposed when working in facilities that produce a variety of lead-containing products. These include radiation shields, ammunition, certain surgical equipment, fetal monitors, plumbing, circuit boards, jet engines, and ceramic glazes. In addition, lead miners and smelters, plumbers and fitters, auto mechanics, glass manufacturers, construction workers, battery manufacturers and recyclers, firing range instructors, and plastic manufacturers are at risk for lead exposure. Other occupations that present lead exposure risks include welding, manufacture of rubber, printing, copper smelting, ore processing, combustion of solid waste and production of paints and pigments. Parents who are exposed to lead in the workplace can bring lead dust home on clothes or skin and expose their children (Dart et al., 2004; Brodkin *et al.*, 2007).

(b) Paints

Some lead compounds are bright colors and are used widely in paints, (Henretig, 2006) andlead paint is a major route of lead exposure in children (Gilbert and Weiss, 2006). Deteriorating lead

paint can produce dangerous lead levels in household dust and soil. Deteriorating lead paint and lead-containing household dust are the main causes of chronic lead poisoning (Salvato, 2003; Pearce, 2007).

(c) Soil

Tetraethyl lead which used to be added to gasoline in the past, caused significant soil contamination. Residual lead in soil contributes to lead exposure in urban areas. Lead content in soil may be caused by broken-down lead paint, residues from lead-containing gasoline or pesticides used in the past, contaminated landfills, or from nearby industries such as smelters (Woolf et al., 2007). Although leaded soil is less of a problem in countries that no longer use leaded gasoline, it remains prevalent, raising concerns about the safety of urban farming (Murphy, 2009). Eating food grown in contaminated soil can present a lead hazard (Yu, 2005).

(d)Water

Lead from the atmosphere or soil can end up in groundwater and surface water (Yu, 2005). It is also potentially in drinking water, e.g. from plumbing and fixtures that are either made of lead or have lead solder (Menkes, 2006). Since acidic water breaks down lead in plumbing more readily, chemicals can be added to municipal water to increase the pH and thus reduce the corrosivity of the public water supply (Chisolm, 2004).

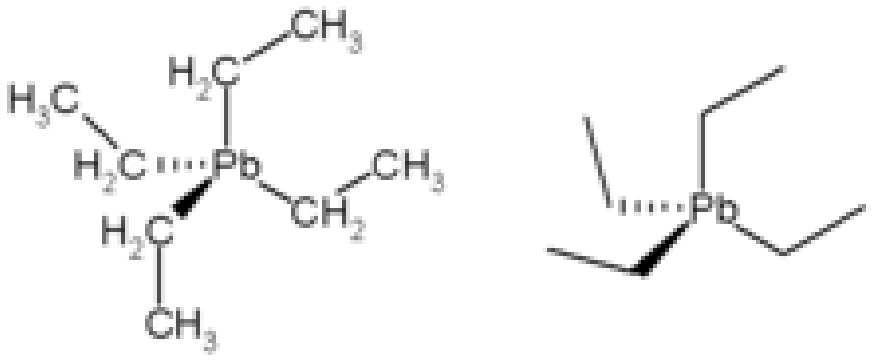


Figure 4: Structure of Tetra ethyl lead . copyright: joaquinbarioso.com/google images

(e) Lead-containing products

Lead can be found in products such as kohl, a South Asian cosmetic, and from some toys (Guidotti and Ragain, 2007). Vinyl mini-blinds, found especially in older housing, may contain lead (Ragan and Turner, 2009). Ingestion of metallic lead, such as small lead fishing lures, increases blood lead levels and can be fatal (Schep *et al*, 2006). Improperly-fired ceramic glaze can leach lead into food, potentially causing severe poisoning (Salvato, 2003). In some places, the solder in cans used for food contains lead. People who eat animals hunted with lead bullets may be at risk for lead exposure (Hunt, *et al*. 2009).

2.5 Pathophysiology of lead poisoning

Lead is stored in body compartments such as the blood, soft tissues, and bone; the half-life of lead in these tissues is measured in weeks for blood, months for soft tissues, and years for bone (Karri and Saper, 2008). Lead in the bones, teeth, hair and nails is bound tightly and not available to other tissues, and may therefore not to be harmful. In adults, 94% of absorbed lead is deposited in the bones and teeth, but children only store 70% in this manner, a fact which may partially account for the more serious health impacts on children The estimated half-life of lead in bone is 20–30 years, and bone can introduce lead into the bloodstream long after the initial exposure is over (Merrill *et al*., 2007). The half-life of lead in the blood in men is about 40 days, but it may be longer in children and pregnant women, whose bones are undergoing remodeling, which allows the lead to be continuously re-introduced into the bloodstream (Barbosa *et al.*, 2005). Also, if lead exposure takes place over years, clearance is much slower, partly due to the continuous release of lead from bone. Many other tissues store lead, but those with the highest concentrations (other than blood, bone, and teeth) are the brain, spleen, kidneys, liver, and lungs.

It is removed from the body very slowly, mostly through urine (Pearson, 2003). Smaller amounts of lead are also eliminated through the feces, and very small amounts in hair, nails, and sweat (Kosnett, 2006).

Lead has no known physiologically relevant role in the body, (White *et al.*, 2007). Lead and other heavy metals create reactive radicals which damage cell structures including DNA and cell membranes (Flora *et al.*, 2008). Anemia may result when the cell membranes of erythrocytes become more fragile as the result of damage to their membranes (Yu, 2005). Lead interferes with metabolism of bones and teeth (Casaret *et al*., 2007), and alters the permeability of blood vessels and synthesis of collagen (Needleman, 2004). Lead may also be harmful to the developing immune system causing increased production of inflammatory proteins. Lead exposure has also been associated with a decrease in activity of immune cells such as polymorphonuclear leukocytes (Casarett et al., 2007). Lead also interferes with calcium metabolism of cells (Chisolm, 2004).

The primary cause of lead's toxicity is its interference with a variety of enzymes due to the fact that it binds to sulfhydryl groups found on many enzymes (Pearson and Schonfeld, 2003). Part of lead's toxicity results from its ability to mimic other metals that take part in biological processes. These metals act as cofactors in many enzymatic reactions, displacing them at the enzymes on which they act (Dart et al., 2004). Lead is able to bind to and interact with many of the same e enzymes as these metals but, due to its differing chemistry, does not properly function as a cofactor, thus interfering with the enzyme's ability to catalyze its normal reaction or reactions. Among the essential metals with which lead interacts are calcium, iron, and zinc (Kosnett, 2006).

One of the main causes for the pathology of lead is that it interferes with the activity an essential enzyme called delta aminolevulinic acid dehydratase. This is an important enzyme in the biosynthesis of heme, the cofactor found in hemoglobin (Patrick, 2006). Lead also inhibits Ferrochelatase, another enzyme involved in the formation of heme (Fujita *et al.*, 2002). Lead's interference with heme synthesis results in the development of anemia (Mycyk *et al.*, 2005). Another effect of lead's interference with heme synthesis is the buildup of heme precursors, which may be directly or indirectly harmful to neurons (Kosnett, 2005).

Lead exposure damages cells in the hippocampus a part of the brain involved in memory (Xu *et al.*, 2009).

Lead affects the release of different neurotransmitters such as glutamate while increasing brain cell apoptosis (Dart *et al*., 2004; Needleman, 2004). The brain is most sensitive to lead exposure (Sanders *et al*, 2009). Lead poisoning interferes with the normal development of a child's brain and nervous system; therefore children are at greater risk of lead neurotoxicity than adults are (Meyer *et al*., 2003). High blood lead levels in adults are also associated with decreases in cognitive performance and with psychiatric symptoms such as depression and anxiety (Shih *et al.*, 2007).

Renal system

Kidney damage occurs with exposure to high levels of lead, and evidence suggests that lower levels can damage kidneys as well (Grant, 2009). The toxic effect of lead causes nephropathy, gout (Shadick *et al.*, 2000), and Fanconi's syndrome (Rubin and Strayer, 2008). This gout is known as saturnine gout.

Cardiovascular system

Evidence suggests lead exposure is associated with hypertension. More studies has found connections between lead exposure and various cardiovascular disease such as coronary heart disease, stroke, etc (Navas-Acien *et al.*, 2007).

Reproductive system

Lead affects both the male and female reproductive systems. In men, when blood lead levels exceed 40 μg/dL, sperm count, sperm volume, motility and morphology are all negatively affected (Grant, 2009). A pregnant woman's elevated blood lead level can lead to miscarriage, prematurity, low birth weight (Cleveland, 2008). Lead is able to pass through the placenta into breast milk, and blood lead levels in mothers and infants are usually similar (Dart *et al.*, 2004). A fetus may be poisoned *in utero* if lead from the mother's bones is subsequently mobilized by the changes in metabolism due to pregnancy; increased calcium intake in pregnancy may help mitigate this phenomenon (Bellinger, 2005).

Diagnosis of lead poisoning

Diagnosis includes determining the clinical signs and the medical history, with inquiry into possible routes of exposure (Fred and van Dijk, 2009). The main tool in diagnosing and assessing the severity of lead poisoning is laboratory analysis of the blood lead levels (Mycyk *et al.*, 2005). Blood film examination may reveal basophilic strippling of erythrocytes (dots in red blood cells visible through a microscope), as well as the changes normally associated with iron-deficiency anemia i.e. microcytosis and hypochromasia (Patrick, 2006; Ekong *et al.*, 2006). Exposure to lead also can be evaluated by measuring erythrocyte protoporphyrin in blood

samples (Patrick, 2006). EP is a part of red blood cells known to increase when the amount of lead in the blood is high, with a delay of a few weeks (Kosnett, 2005). However, due to the higher threshold for its detection, use of this method for detecting lead exposure has decreased (Vaziri, 2008).

Fecal lead content that is measured over the course of a few days may also be an accurate way to estimate the overall amount of childhood lead intake. This form of measurement may serve as a useful way to see the extent of oral lead exposure from all the diet and environmental sources of lead (Payne, 2008).

Prevention of lead poisoning

In most cases, lead poisoning is preventable (Watts, 2009) the way to prevent it is to prevent exposure to lead (Rossi, 2008). Prevention strategies can be divided into individual (measures taken by a family), preventive medicine (identifying and intervening with high-risk individuals), and public health (reducing risk on a population level) (Guidotti and Ragain, 2007).

Recommended steps by individuals to reduce the blood lead levels of children include increasing their frequency of hand washing and their intake of calcium and iron, discouraging them from putting their hands to their mouths, vacuuming frequently, and eliminating the presence of lead-containing objects such as blinds and jewellery in the house (Chisamera, 2008). In houses with lead pipes or plumbing solder, these can be replaced (Chisamera, 2008). Less permanent but cheaper methods include running water in the morning to flush out the most contaminated water, or adjusting the water's chemistry to prevent corrosion of pipes (Chisamera, 2008). Lead testing kits are commercially available for detecting the presence of lead in the household (Hunt *et al*, 2009).

Screening is an important method in preventive medicine strategies (Guidotti and Ragain, 2007).

Recommendations by health professionals for lowering childhood exposures include banning the use of lead where it is not essential and strengthening regulations that limit the amount of lead in soil, water, air, household dust, and products (Pokras and Kneeland, 2008).

Treatment of lead poisoning

Table 1: Center for Disease Control and Prevention management guidelines for children with elevated blood lead levels (Henretig, 2006).

Blood lead level (µg/dL)	**Treatment**
10–14	Education, repeat screening
15–19	Repeat screening, case management to abate sources
20–44	Medical evaluation, case management
45–69	Medical evaluation, chelation, case management
>69	Hospitalization, immediate chelation, case management.

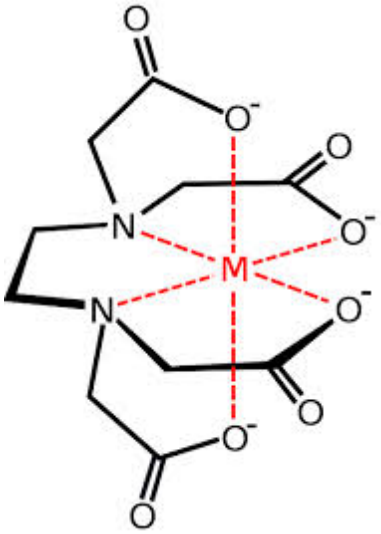


Figure 5: Structure of ethylenediaminetetraacetic acid. Copyright: whatscookingwithdoc.com/google images

Epidemiology of lead poisoning

Since lead has been used widely for centuries, the effects of exposure are worldwide (Payne, 2008). Environmental lead is ubiquitous, and everyone has some measurable blood lead level (Hu *et al*, 2007). Lead is one of the largest environmental medicine problems in terms of numbers of people exposed (Pokras, and Kneeland, 2008). Although regulation reducing lead in products has greatly reduced exposure in the developed world since the 1970s, lead is still allowed in products in many developing countries (Pokras, and Kneeland, 2008). In all countries that have banned leaded gasoline, average blood lead levels have fallen sharply (Meyer *et al.*, 2008). However, some developing countries still allow leaded gasoline (Payne, 2008) which is the primary source of lead exposure in most developing countries (Meyer *et al*., 2003). Poor children in developing countries are at especially high risk for lead poisoning (Payne, 2008).

In developed countries, non-white people with low levels of education living in poorer areas are most at risk for elevated lead (Pokras and Kneeland, 2008). Low-income people often live in old housing with lead paint, which may begin to peel, exposing residents to high levels of lead-containing dust.

Risk factors for elevated lead exposure include alcohol consumption and smoking (possibly because of contamination of tobacco leaves with lead-containing pesticides) (Hu *et al* 2007). Adults with certain risk factors might be more susceptible to toxicity; these include calcium and iron deficiencies, old age, disease of organs targeted by lead (e.g. the brain, the kidneys), and possibly genetic susceptibility (Kosnett *et al*, 2007). Differences in vulnerability to lead-induced neurological damage between males and females have also been found, but some studies have found males to be at greater risk, while others have found females to be (Bellinger, 2004).

In adults, blood lead levels steadily increase with increasing age (Trevor *et al.*, 2007). In adults of all ages, men have higher blood lead levels than women. Children are more sensitive to elevated blood lead levels than adults are (Murata *et al.*, 2009). Children ages one to three tend to have the highest blood lead levels, possibly because at that age they begin to walk and explore their environment, and they use their mouths in their exploration (Bellinger, 2004). Blood levels usually peak at about 18–24 months old (Pearson, 2003).

Blood lead reference values

The current reference range for acceptable blood lead concentrations in healthy persons without excessive exposure to environmental sources of lead is less than 10 µg/dL for children and less than 25 µg/dL for adults (Wu, 2006). The current biological exposure index (a level that should not be exceeded) for lead-exposed workers in the U.S. is 30 µg/dL in a random blood specimen. Blood lead concentrations in poisoning victims have ranged from 30-80 µg/dL in children exposed to lead paint in older houses, 77-104 µg/dL in persons working with pottery glazes, 90-137 µg/dL in individuals consuming contaminated herbal medicines, 109-139 µg/dL in indoor shooting range instructors and as high as 330 µg/dL in those drinking fruit juices from glazed earthenware containers (Baselt, 2008).

2.7 Lead poisoning in Nigeria

Between 2010 and 2012, a series of lead poisonings in Zamfara State, Nigeria has led to the deaths of more than 400 people. More than 6,000 people were affected in the surrounding areas. The Federal ministry of Health puts the fatality rate at 46% (Sahabi, 2010). This is arguably the worst lead poisoning epidemic in modern times.

The poisoning was caused by gold mining. An investigation showed that people had been digging for gold at the times of their deaths, in an area where lead is prevalent. The villagers thought that the children had contracted malaria but doctors found unusually high levels of lead in the blood during tests. Blacksmith Institute was called in by the Federal Government of Nigeria to help in the removal of toxic lead.

Illegal mining for gold by the villagers made them to take home gold ores where they are crushed to extract gold. This results in the soil being contaminated from lead which then poisons people through hand-to-mouth contamination. Others have been contaminated by contact with contaminated tools and water. Illicit gold mining is more lucrative than agriculture for the people of these communities.

In an effort to halt the lead poisoning, the authorities are clamping down on illegal mining and carrying out a clean-up of the area so that surviving children returning from treatment will not be re-exposed to toxic lead in their homes (Sahabi, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental animals

Two hundred and forty (240) healthy male albino rats of Wistar strain weighing between 80 and 100 grams were used for the study. These animals were divided into 3 groups of 80 rats each. The animals were housed under standard conditions of temperature (23±2ºC), humidity (55±15%) and 12hr light (7.00am-7.00pm) in the animal house of Department of Physiology, University of Ibadan. They were kept in wire meshed cages with beddings, which were adequately changed throughout the period of study. Animals were allowed to acclimatize to animal house conditions for two weeks with free access to commercial rat chow and tap water *ad libitum* before commencement of studies.

3.1.1 Grouping of animals

The animals were randomly divided into three groups: **control** (distilled water), **low-dose** (100 ppm) of lead acetate dissolved in drinking water for 20 weeks; and **high-dose** (5,000 ppm) of lead acetate dissolved in drinking water for 20 weeks groups.

3.2 Chemicals and reagents

1. Lead acetate (99%, BDH Chemicals Ltd Poole, England).
2. Concentrated acetic acid (99%)
3. Sodium chloride (99.9%, BDH Chemicals Ltd Poole, England).
4. Thiopental sodium (Rotex Medica, Germany)
5. Histamine acid phosphate (BDH Chemicals, UK)
6. Diethyl ether
7. Urethane 99.2% (BDH Chemicals Ltd Poole, England)
8. Methylated spirit
9. Chloroform
10. Caspase-3 substrate Ac-DEVD-pNA (10mM)
11. Equilibration buffer
12. Biotinylated nucleotide
13. Terminal deoxynucleotidyl transferase recombinant
14. 10mg proteinase K
15. Streptavidin HRP (0.5mg/ml)
16. DAB 20X chromogen
17. DAB substrate 20X buffer

18. Hydrogen peroxide 20X

19. DeadEnd colorimetric TUNEL system

20. Phosphate-buffered saline (PBS)

21. 0.3% hydrogen peroxide.

22. Fixative

23. 0.2% Triton X-100 solution in PBS

24. Xylene

25. Ethanol (100%, 95%, 85%, 70% and 50%) diluted in deionized water

26. 0.85% NaCl solution

27. Proteinase K buffer

28. Phosphate buffered saline (PBS)

29. Mounting medium

30. Poly-L-lysine-coated microscope slides,

31. Deionized water

32. indomethacin

33. Others

3.3 **Equipment and Materials**

1. Forceps
2. Coplin jars
3. Humidified chambers
4. 37°C incubator
5. Micropipettors
6. Glass coverslips
7. Parafilm laboratory film
8. Clear nail polish or rubber cement
9. Microscope
10. Eye forceps
11. Plastic Coverslips
12. Others

3.4 Procedure of chronic lead treatment

The method of Gruber *et al.,* (1977) was used with slight modifications. Animals were treated with lead acetate dissolved in their drinking water at 100 ppm and 5,000 ppm for low-dose and high-dose groups respectively for 20 weeks and fed with commercial rat pellets. The control animals drank normal tap water. All animals were allowed access to rat chow and their drinking water *ad libitum.*

3.5 Induction of ulcer

(a) Acetic acid method

At the end of 20 weeks, ulcer was induced by the method of Wang *et al.,* (1989) with slight modifications. Food was withheld 24-36hrs before ulcer induction, but with access to water. The animals were anaesthesized with Thiopental sodium (50mg/Kg) and laparatomy was performed to expose the stomach. Acetic acid (0.5mL, 60% vol/vol) was applied to the serosal surface of the glandular portion of the stomach for 1 minute using a 3mL syringe barrel which had been cut and smoothed. The acid was then removed by aspiration and the area washed with sterile saline and dabbed with cotton wool. The abdomen was sutured and the animals returned to their diets and water. The animals were sacrificed at intervals to check for ulcer healing (i.e. on days 0, 7, 14, and 21 post-ulcer induction).

(b) Indomethacin method

Animals were fasted for 24 hours but with access to water before they were administered with 40mg/kg body weight indomethacin. At 24 hours, the animals were sacrificed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored according to the method described by Elegbe and Bamgbose (1974) and modified by Ibironke *et al.*, (1997).

3.6 Experimental procedures

The experiments were divided into 6 studies as follows:

Study 1 - Preliminary study on effects of chronic lead exposure on body weight changes and plasma lead Levels in male albino Wistar rats:

a. Measurement of weekly body weight changes in the animals

b. Measurement of plasma lead (Pb) levels in all animal groups

Study 2 – Measurement of ulcer dimensions in animals chronically exposed to lead:

a. By planimetry

b. By histomorphometry

c. By gross macroscopic scoring

Study 3 – Assessment of the effects of lead exposure on the characteristics and quality of ulcer healing:

a. By histological method

b. By histomorphometry

Study 4 – Assessment of the effects of lead exposure on gastric acid secretion during ulcer healing:

a. By measuring the basal gastric acid secretion
b. By measuring the peak histamine-stimulated gastric acid secretion

Study 5 – Assessment of the effects of lead exposure on blood cells and plasma biochemical parameters during ulcer healing:

a. Estimation of haematological indices

b. Estimation of plasma electrolytes levels

Study 6 – Assessment of the effects of chronic lead exposure on cellular activities during ulcer healing:

a. By biochemical study (Catalase, SOD and MDA)

b. By assessment of Apoptosis – TUNEL assay

3.6.1. Study 1: Preliminary studies-body weight changes and determination of plasma lead levels

a. Measurement of weekly body weight changes of the animals

Weekly changes in body weight were measured and recorded using digital weighing scale (Citizen Model MP, 2000).

b. Measurement of plasma lead levels in all groups

Plasma lead levels were determined using spectrophotometric techniques (digital atomic absorption flame spectrophotometer) at the Multidisciplinary Central Research Laboratories, University of Ibadan.

3.6.2 Study 2-Measurement of ulcer dimensions

a. By planimetry

The animals were sacrificed on days 0, 7, 14 and 21 post ulcer-induction and the stomachs were removed, opened along greater curvature, rinsed with normal saline and pinned on a wax block. Transparent paper was placed over ulcer area and traced out. The area of ulceration was converted to units of square millimeters using 1mm by 1mm paper grid. The rate of healing on day 14 was calculated as:

(Ulcer area on day 14-ulcer area on day 7)/ulcer area on day 14.

While rate of healing per day on day 21 was calculated as:

(Ulcer area on day 21-ulcer area on day 7)/ulcer area on day 21

b. By histomorphometry

A graticle having 1 to 100µm calibration was attached to a microscope, using the histological slides that was previously prepared, ulcer depth and width were measured and recorded, the mucosal area eroded was then calculated.

c. By gross macroscopic scoring.

The stomachs were removed and opened along the greater curvature. It was rinsed with normal saline. The severity of damage done to the inner surface (mucosal lesions) was observed macroscopically and scored as described by Ibironke et al (1997) as follows: pin point-0.5, laceration/2 or more -1, laceration/>3mm -2, deep cut -3.

3.6.3 Study 3 – Assessment of the characteristics and quality of healing

a. By histological method

Histological studies were performed according to the methods described by Oghiara and Okabe (1993). At autopsy, the ulcers were embedded in paraffin and sectioned at 5 micrometers in an automated microtome. Haematoxylin and eosin and PAF stainings were done. Tissue contraction and regeneration of the ulcerated mucosa and inflammatory exudates were observed under stereomicroscope. Digital photomicrographs were also taken. The tissues were allowed to fix in 10% formal saline for 48 hours. Then they were grossed and cut into smaller pieces 3mm thick in prelabelled tissue cassette. They were processed using automatic tissue processor (LEICA TP1020) where they passed through various reagents including alcohol (of various concentrations starting from 70%, 80%, 90%, 95%, 100%,100%) for dehydration, two changes of xylene and three changes of molten Paraffin wax set at 65 degree centigrade. The processing time was 12 hours.

The tissues were then embedded in wax, forming paraffin blocks, ready for microtomy. Next, they were sectioned at 4 microns using rotary microtome (LEICA R T2115). The sections were floated on hot water bath to attach the sections to prelabelled slides. The sections were dried on hot plate and made ready for staining using haematoxylin and eosin staining techniques.

Haematoxylin and eosin staining techniques:

The following were the steps involved in haematoxylin and eosin staining:

1.The tissue sections were taken to water.

2. The sections were stained in harris haematoxylin for 5 minutes.

3. Next, they were rinsed in water.

4. They were briefly differentiated in 1% acid alcohol.

5. They were rinsed and blued under tap water for 10 minutes.

6. Next, they were counterstained in 1% aqueous eosin for 3 minutes and rinsed in water.

7. The sections were dehydrated through ascending grades of alcohol (70%, 80%, 90% and absolute).

9. Finally, they were cleared in xylene and mounted with DPX mountant.

Procedure for periodic acid schiff reaction staining:

1. The tissue sections were taken to water.

2. They were oxidized in 1% periodic acid for 5 minutes and rinsed in distilled water.

3. Schiff s reagent was applied to tissue sections for 15 minutes and rinsed in distilled water.

4. The nuclei were counterstained with Mayers haematoxylin for 2 minutes and rinsed in water.

5. Tissues were dehydrated in alcohol, cleared in xylene and mounted.

b. By histomorphometry

Histomorphometric studies were done at the Department of Veterinary Anatomy, University of Ibadan. A graticle with 1-100µm calibration was attached to the microscope. Using the histological slides previously prepared, fibroblast, parietal and mucous cells numbers and dimensions were estimated under the microscope.

3.6.4 Study 4 – Assessment of gastric acid secretion during ulcer healing in chronically lead exposed rats.

a. By measuring the basal and histamine stimulated gastric acid secretion

i. Surgical preparation of animals

Each animal was weighed. They were then anaesthetised with 25% urethane injected intraperitoneally at a dose of 0.6 mL/100g body weight. The animal limbs were tied to a dissecting board. A blunt dissection was done in the neck region and the trachea was exposed. The oesophagus was separated from the trachea by using a curved forceps to free them from each other. Threads were passed between the two structures. A small cut was made in the upper part of the trachea and a 2.5cm long polythene size 3 cannula was inserted into it. The thread below the trachea was then tied lightly round the inserted cannula. The significance of trachea cannulation was to avoid any respiratory difficulties, by-passing the nasal passage to the larynx. Also with the cannula in place, any fluid accumulating in the trachea can be readily aspirated. Also the essence of exposing the oesophagus before cannulating the trachea was to prevent puncturing the esophagus during the passage of esophageal cannula from the mouth. To prevent drying up of the exposed tissue, saline soaked cotton wool was placed over the dissected area.

Another size 3 polythene cannula from a Lagendorff apparatus was passed into the oesophagus to reach the cardia region of the stomach.

The linear alba was cleared of hair, a midline incision was made through the skin and muscle of the abdomen. The stomach was brought out, a small cut was made about an inch distal to the pyloroduodenal junction, the perfusion fluid maintained at 37 ^{o}C was rushed from the Lagendorff apparatus tap to wash out all food debris from the stomach of the animal. With the perfusion

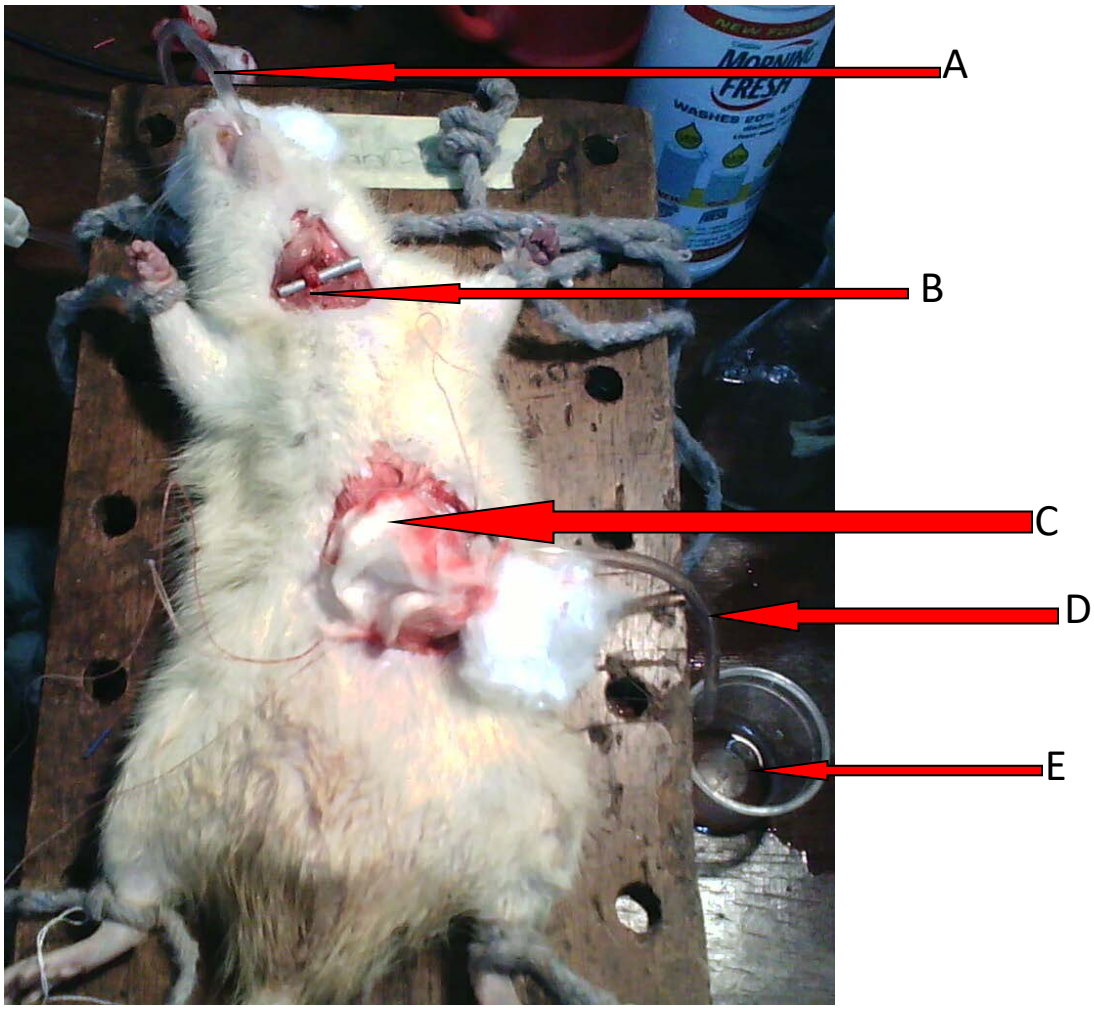


Plate 1: Set-up of cannulated rat during gastric acid secretion study.

Arrows are pointing at (starting from the top down): A- oesophageal cannula, B - exposed trachea, C- cotton wool soaked in saline, D- duodenal cannula and E - vessel for collecting gastric secretion.

fluid still running, the early part of the duodenum was cannulated and the stomach was packed back into the peritoneum. The muscle was sutured back before the skin. It was ensured that the perfusion fluid was running out of the stomach. After collecting consistent basal output, 0.5mg/kg histamine acid phosphate was injected into each animal intramuscularly.

ii. Collection of samples

The rate of flow of the perfusing fluid was manually adjusted such that 10 mL of effluent (which contains gastric juice from the stomach) was collected at 10 minute intervals. The first few effluents (4-5) were collected to establish a consistent basal output.

iii Titration procedure

After each sample collection, 5 mL of the 10mL collected was measured into a conical flask and two drops of phenolphthalein reagent was added. 0.0025M NaOH was run dropwise into the cornical flask, shaking the flask in the process until the first appearance of a pink colour. After this, the titration was stopped and the end point recorded.

iv. Calculation of acidity

The reaction between gastric acid and the titrating base can be represented by the equation below:

$NaOH + HCl \rightarrow NaCl + H_2O$ ……(1)

At the end-point of titration

$N_A V_A = N_B V_B$

i.e. $N_A = \frac{N_B x V_B}{V_A}$ ……..(2)

But normality (N) = Concentration (C) in gm/litre

Gm. Eq. Wt (G) i.e $C = N \times G$

(Acid concentration is gm/litre (C) = $N_A \times G$

Substituting for N_A in equation 2,

$C = \frac{N_B . V_B . G}{V_A}$ gm/litre

$C = \frac{N_B . V_B G}{V_A} \times 100$ mg/100ml ..……..…(3)

But acid concentration is meq/litre – Concentration in mg/litre (m. Eq. Wt. (G)

Therefore, Acid concentration in Meq/litre

$= \frac{N_B . V_B . G}{V_A . G} \times 100$……(4)

But $N_B = 1/800$

Substituting for N_B in (equation 3)

Acid concentration in Meq/litre $= 1/800 \times V_B\ 1000 = 5/4\ V_B.$

Since the gastric effluent sample titrated =VA = 5ml.

The acid concentration titrated sample (Meq/litre) = V_B

2 ……..(5)

But the 5ml is only ½ of the total effluent sample

Therefore, total acidity for each 10ml sample = $\frac{V_B \times 2}{4} = \frac{V_B}{2}$

3.6.5 Study 5 – Assessment of the effects of blood cells and plasma biochemical parameters during ulcer healing

a. **Estimation of haematological indices**

b. **Estimation of serum electrolytes**

a. Estimation of haematological indices

Packed cell volume estimation

A plane capillary tube was filled with blood to about three-quarter its length. The end of capillary tube free of blood was sealed off with plasticine. The tube was spinned in a haematocrit centrifuge for 20 minutes at 3000 rpm. The height of the column of packed red cells was read and the result was expressed as the percentage of volume of red cells to whole column of blood.

Red blood cell count

Blood was sucked into red blood cell pipette up to 0.5 mark, holding the pipette more or less horizontal during the process. Hayem's solution was drawn to the 101 mark, care was taken not

to over shoot and rotating the pipette while doing so. The rubber tubing was detached, mixed thoroughly for 1minute by vigorous agitation of the pipette held between the thumb and middle finger. The tip of pipette was brought in contact with exposed part of the counting chamber, the pipette was raised towards vertical position until diluted blood flowed under the cover slip. The counting chamber was placed on the horizontal stage of the microscope, the corpuscles were allowed to settle for 2 minutes.

Using x 40 objective lens of the microscope, the number of corpuscles in each of 80 small squares i.e. 5 sets of small squares was counted. All corpuscles overlapping the top and left hand sides of the square were counted while those overlapping the bottom and right side were not counted to avoid counting twice.

Calculation:

Volume of diluted blood over each small square 1/4000 cubic mm (since depth of the chamber is 1/10mm and area is 1/400 square mm). Volume of diluted blood over 80 small squares 80/4000 cubic mm. Let X be the number of corpuscles lying over 80 small squares, then X corpuscles were present in 80/4000 cubic mm of diluted blood. Blood is diluted 1 to 200.

Hence 80/4000 cubic mm of the (undiluted) blood contains 4000/80 corpuscles and 1 cubic .mm of the (undiluted) blood contained:

$$\frac{4000X \times 200}{80} = 10000N \text{ corpuscles}$$

White blood cells count

From the blood collected, 20µL of blood was added to 0.38mL of Tuerk's solution in a test tube, to give a 1:20 dilution. After mixing, a Pasteur pipette was used to draw the mixture and the counting chamber was charged. The counting chamber was placed on the horizontal stage of a microscope, the corpuscles were allowed to settle for 2 minutes.

Using x 10 objective lens of the microscope, the number of corpuscles in the four large squares was counted. All corpuscles overlapping the top and left hand sides of the square were counted while those overlapping the bottom and right side were not counted to avoid counting twice.

Calculation

N x 50 cells/mm^3

Differential white blood cell count

A drop of blood was added to one end of a slide, the slide was quickly placed on the bench, holding it in position with the thumb and the index finger on the left hand. The narrow end of the second slide is then placed in the drop and held there until the blood has spread across it is then drawn slowly over the whole length of the first slide, being held meanwhile at an angle of 45^o . After the blood has spread, it was dried by waving the slide rapidly in air to prevent undue shrinkage of the cells.

The film was stained with Leishman stain for 10 minutes, washed in a gentle stream of water. Dried with filter paper and examined under low and high power. With a properly stained film, the red cells were pink, the cytoplasm of white cells faintly blue. The small neutrophil granules were brick red and the basophils dark blue.

A table was made headed with the different varieties of white cells. Blood cells (200) were counted, marking each as it was recognized in the appropriate column of the table. The percentage of each type present was calculated.

Platelets

Blood (20µL) was added to 0.38mL Boar's fluid in a test tube, giving a 1:20 dilution. After mixing, a Pasteur pipette was used to draw mixture and the counting chamber was charged. The counting chamber was placed on the horizontal stage of the microscope. The platelets were allowed to settle for 2 minutes.

Using x 40 objective lens of the microscope, the number of platelets in each of 80 small square i.e 5 sets of small squares was counted. All platelets overlapping the top and left hand sides of the square were counted while those overlapping the bottom and right side were not counted to avoid counting twice.

Calculation

N x 1000cell/mm3

b. Estimation of plasma electrolytes

Plasma electrolytes were estimated using ion selective electrode method (ISE) (Easylite Analyser) and diacetyl monoxime method (for urea) at the radioimmunoassay laboratories of Chemical Pathology Department, University College Hospital, Ibadan.

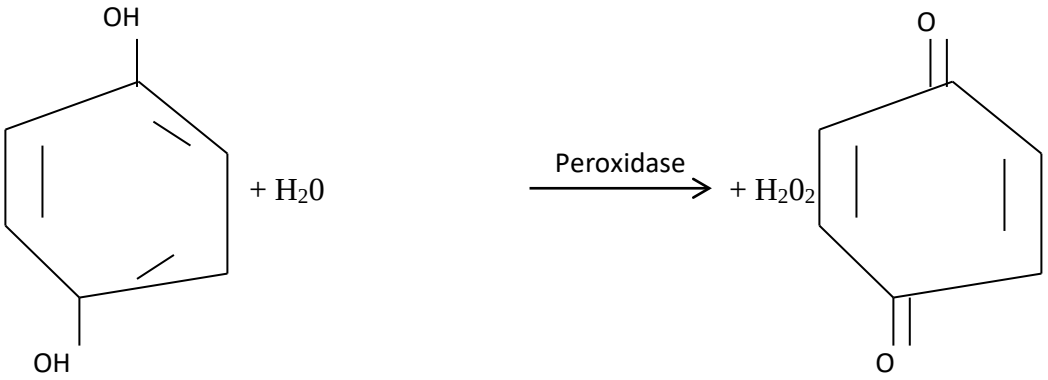
3.6.6 Study 6- Biochemical study

a. Determination of catalase activity

Principle: Catalase is present in nearly all animal cells, plants and bacteria and acts to prevent accumulation of noxious H_2O_2 which is converted to O_2 and H_2O.

i.e. $H_2O_2 + H_2O_2 \leftrightarrow 2H_2O + O_2$

Peroxidase which is less widely distributed, catalyzes the following reaction:



Procedure:

Three test tubes were labeled as sample, blank and spectrophotometer standard respectively. Half a milliliter of the sample (tissue homogenate) was added to the first test tube only. Next, 5mL of 30Nm H_2O_2 was added to sample and blank test tubes. Next, 0.5mL of distilled water was added to blank. The contents of the test tubes were mixed by inversion and allowed to stand for 3 minutes each. Next, 1mL of 6M H_2SO_4 was added to all 3 test tubes and 5.5mL of 0.05M Phosphate buffer (pH7.4) to the third test tube only. All test tube contents were mixed by inversion again. Next, 7 mL of 0.01M $KMnO_4$ solution was added to all test tubes and mixed by

inversion. Absorbance was read at 480nm within 30 – 60 seconds against distilled water using a spectrophotometer.

Calculation:

Absorbance of blank = Abs_B

Absorbance of spectrophotometer standard = Abs_{std}

Absorbance of test = Abs_T

$$K_c log = \frac{S_o \text{ X } 203}{S_3 . \ t}$$

$S_O = Ab_{std} - Abs_B$

$S_3 = Abs_{std} – Abs_T$

S_O = [S] at zero time

S_3 = [S] at t = 3 minutes

$$K_C = \frac{\text{inverse Log of } S_O \text{ X } 203}{S_3 . \ t}$$

b. Determination of superoxide dismutase activity:

This was determined according to the method of Misra and Fridovich (1989).

Principle**:**

Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome, whose concentration can be determined at 420nm using a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions. The enzyme SOD inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anion. The degree of inhibition is thus a reflection of the activity of SOD and it is determined as one unit of the enzyme activity.

Reagents**:**

i) ***0.05M Carbonate buffer (pH 10.2).*** This was prepared by dissolving 0.2014g of Na_2CO_3 and 0.0372g of EDTA in about 80mL distilled water, and after adjusting the pH to 10.2 with 1M NaOH, the volume was made up to 100mL with distilled water.

ii) ***0.01mM Adrenaline solution***. This was prepared by dissolving 0.01098g of adrenaline in 200mL of 0.005M HCl solution. This was prepared fresh.

iii) ***0.005 HCl solution:*** This was prepared by diluting 5mL of 0.IM HCl solution to 100mL with distilled water.

iv) ***0.10 M HCl solution***: This was prepared by diluting 1mL of commercial concentrated HCl to 120mL with distilled water.

Procedure:

Having set the spectrophotometer at 420nm, it was zeroed with a blank made up of 3.0mL of distilled water. 0.2mL of distilled water was added to the reference tube while 0.2mL of the appropriate enzyme extracts were added to appropriate labeled test tubes. To each of these was added 2.5mL of carbonate buffer, followed by equilibration at room temperature. 0.3mL of

0.3mM adrenaline solution was then added to the reference and each of the test solutions, followed by mixing and reading of absorbance at 420nm.

Calculation:

$$\text{Inhibition} = \frac{\text{O.D.Ref - O.DTest x100}}{\text{O.D. Ref.}}$$

However, 1 unit of SOD activity is taken as the amount of SOD required to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome per minute. So, the enzyme activity can be calculated as follows:

$$\text{Units/mg of wet tissue} = \frac{\text{\% Inhibition}}{50 \times y}$$

Where y = mg of tissue in the volume of the sample used.

c. Estimation of malondialdehyde content (lipid peroxidation).

The assay method of Hunter *et al.,* (1963), modified by Gutteridge and Wilkins (1980), was adopted.

Principle: Malondialdehyde (MDA, a product of lipid peroxidation), when heated with 2-thiobarbituric acid (TBA) under acid condition forms a pink coloured product which has a maximum absorbance at 532nm.

Reagents:

i) Glacial acetic acid.

ii) 1% Thiobarbituric acid (TBA): This was prepared by dissolving 1g of thiobarbituric acid in 100mL of 0.2% NaOH solution.

iii) 2.0% NaOH in 100mL of distilled water

Procedure**:**

3mL each of glacial acetic acid and 1% TBA solution were added to test tubes appropriately labeled blank and tests. 0.6mL of distilled water was added to the blank, while 0.6ml of the appropriate tissue extract was added to each of the "test" tubes. All test tubes were thoroughly mixed, incubated in a boiling water bath for 15 minutes and then allowed to cool. Next, they were centrifuged and their supernatants collected. The supernatant from the blank was used to zero the spectrophotometer. (The spectrophotometer was preset at 532nm). Finally, absorbance of the supernatants from the test solutions was read from the spectrophotometer.

Calculation:

Concentration of MDA in the tissue (moles MDA/g wet tissue)

$$= \frac{0.D \times V \times 1000}{A \times v \times l \times y}$$

Where

0. D = Absorbance of test at 532nm.

V= Total volume of the reaction mixture = 6.6mL

A= Molar extinction coefficient of the product and according to Buege and Aust, (1978) is equal to **1.56×10^5 m^{-1} cm^{-1}**

l = Length of light path =1cm.

y= mg of tissue in the volume of the sample used.

v=Volume of tissue extract used =0.6mL

a. Determination of apoptosis using TUNEL assay

The dead end colorimetric TUNNEL system G7130 and G7360 (Apoptosis test kits) were obtained from Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399 USA. The DeadEnd Systems are nonradioactive systems designed for specific detection and quantitation of apoptotic cells within a population consisting of both apoptotic and non-apoptotic cells. Using the DeadEnd™ Systems, the fragmented DNA of apoptotic cells is end-labeled using a modified TUNEL (TdT-mediated dUTP Nick-End Labeling) assay.

In the deadend Colorimetric System, biotinylated nucleotide is incorporated at the 3′-OH DNA ends using the enzyme terminal deoxynucleotidyl transferase (TdT). Horseradish-peroxidase-labeled streptavidin (Streptavidin HRP) is then bound to these biotinylated nucleotides, which are detected using the peroxidase substrate, hydrogen peroxide, and the stable chromogen, diaminobenzidine (DAB). Using this procedure, apoptotic nuclei are stained dark brown. The system can be used to assay apoptotic cell death in both tissue sections and cultured cells by measuring nuclear DNA fragmentation, an important biochemical indicator of apoptosis in many cell types.

Most cells from higher eukaryotes have the ability to self-destruct by activation of an intrinsic cellular suicide program referred to as programmed cell death or apoptosis (Ellis *et al*., 1991; Steller, 1995). Apoptosis is important in development, homeostasis and several diseases (Burek and Oppenheim, 1996). It is characterized by certain morphological features, including membrane blebbing, nuclear and cytoplasmic shrinkage and chromatin condensation. Cells undergoing apoptosis fragment into membrane-bound apoptotic bodies that are readily phagocytosed and digested by macrophages or neighboring cells without generating an inflammatory response.

Procedure for induction of apoptosis in tissue sections:

1. The tissue samples were prepared by attaching them to a microscope slide, fixing the samples, washing and permeabilizing them with 0.2% Triton X-100 in PBS.
2. The slides were pre-equilibrated with equilibration buffer.
3. DNA strand breaks were labeled with biotinylated nucleotide mix (60 minutes at 37°C).
4. The reaction was stopped by immersing the slides in 2X SSC (15 minutes at room temperature).
5. The slides were washed three times for 5 minutes each in PBS.
6. Hydrogen peroxide was used to block the reaction (3–5 minutes at room temperature).
7. The slides were washed again 3 times for 5 minutes each in PBS.
8. Streptavidin HRP diluted in PBS was added for 30 minutes at room temperature.
9. The slides were washed 3 times for 5 minutes each in PBS.

10. Next, DAB was added and the color developed for approximately 10 minutes.

11. Finally, the slides were rinsed several times in deionized water and analyzed with a light microscope.

Procedure for the analysis of apoptosis in tissue sections:

1. The tissue sections were deparaffinized by immersing the slides in fresh xylene in a coplin jar for 5 minutes at room temperature. This was repeated.

2. Tissue sections were washed by immersing the slides in 100% ethanol for 5 minutes at room temperature in a coplin jar.

3. The 100% ethanol wash was repeated, this time for 3 minutes only. Tissue sections were rehydrated by sequentially immersing the slides through graded ethanol washes (95%, 85%, 70% and 50%) for 3 minutes each at room temperature.

4. They were washed by immersing the slides in 0.85% NaCl and PBS for 5 minutes each at room temperature.

5. The tissue sections were fixed by immersing the slides in 10% buffered formalin in PBS for 15 minutes at room temperature.

6. The sides were immersed in PBS for 5 minutes at room temperature twice.

7. The liquid from the tissue was removed and the slides were placed on a flat surface. 20µg/mL Proteinase K solution was prepared from the 10mg/ml Proteinase K stock solution by diluting

1:500 in PBS. 100µL of the 20µg/ml Proteinase K solution was added to each slide to cover the tissue sections and they were incubated for 10-30 minutes at room temperature.

8. The slides were washed by immersing them in PBS for 5 minutes at room temperature in a coplin jar.

9. The tissue sections were refixed after washing by immersing the slides in 10% buffered formalin in PBS for 5 minutes at room temperature.

10. The slides were washed by immersing in PBS for 5 minutes at room temperature.

11. Step 10 above was repeated.

12. Excess liquid was removed by tapping the slides. The tissue sections were covered with 100µL of equilibration buffer to equilibrate at room temperature for 5-10 minutes.

13. While the sections are equilibrating, i thawed the biotinylated nucleotide mix on ice and prepared sufficient rTdT reaction mix for all experimental and control reactions.

14. Tissue paper was used to blot around the equilibrated areas and 100µL of rTdT reaction mix was added. The sections were not allowed to dry.

15. The sections were covered with plastic coverslips to ensure even distribution of the reagent. The slides were incubated at 37°C for 60 minutes inside a humidified Chamber to allow the end-labeling reaction to occur.

16. The 20X SSC was diluted 1:10 with deionized water. The reactions were terminated by immersing the slides in 2X SSC in a coplin jar for 15 minutes at room temperature.

17.The slides were washed by immersing them in fresh PBS for 5 minutes at room temperature twice.

18. Endogenous peroxidases were blocked by immersing the slides in 0.3% hydrogen peroxide in PBS for 3-5 minutes at room temperature. The slide were washed thrice by immersing them in PBS for 5 minutes at room temperature.

20. The Streptavidin HRP solution was diluted 1:500 in PBS. 100µL was added to each slide

and incubated for 30 minutes at room temperature.

21. The slides were washed by immersing in PBS for 5 minutes at room temperature three times..

22. The DAB components were combined just prior to use by adding 50µL of the DAB Substrate 20X Buffer to 950µL deionized water and 50µL of the DAB 20X Chromogen and 50µL of Hydrogen Peroxide 20X to make the DAB solution. 100µL of DAB solution was added to each slide and developed until there was a light brown background.

23. The slides were rinsed several times in deionized water and mounted in permanent mounting medium.

24. A light microscope was used to observe staining and score apoptosis.

3.7 Statistical analysis

Data obtained were expressed as Mean and standard error of mean (Mean ± SEM). Data were analyzed using descriptive statistics and Student's t-test. The difference between the means was determined using independent sample Students t-test. P values less than or equal to 0.05 were considered significant using Student t-test.

CHAPTER FOUR

RESULTS

4.1 Body weight changes in control and lead exposed animals.

Presented in Figures 5 and 6 are the effects of 20 weeks lead exposure on percentage weight gained by different groups of animals and weekly body weight changes in rats. The weight gained in the low dose group was reduced by 22% of the control value. A further significant reduction to 26% of control values was observed in the high dose group ($P<0.05$).

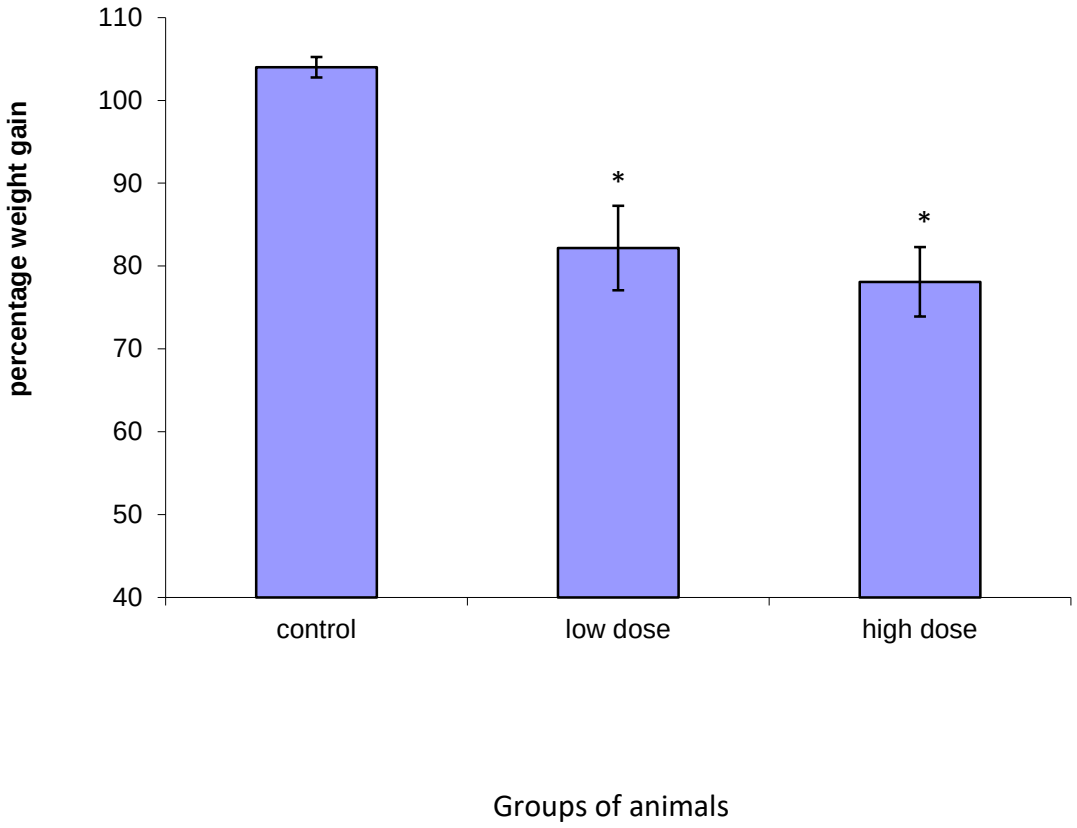


Figure 5: Effects of chronic lead exposure on final percentage body weight gained in rats

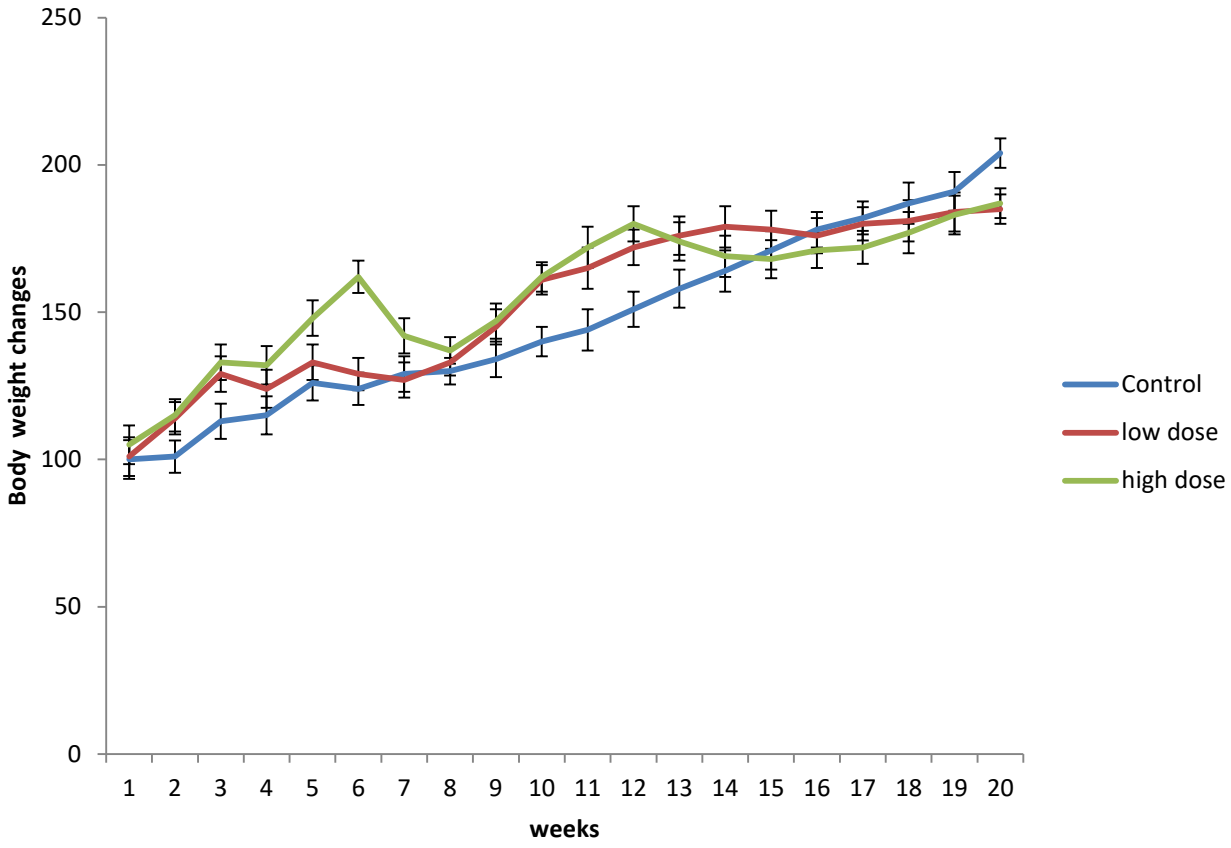


Figure 6: Effects of chronic lead exposure on weekly body weight changes in rats

4.2 Plasma lead levels in rats after 20 weeks of lead treatment.

Shown in figure 7 is the effect of 20 weeks lead exposure on plasma lead levels in control and lead treated rats. The plasma lead level in the low lead (low dose) group increased more than five folds (560% of the control value). A ten-fold increase (990% of control values) was observed in the high dose group compared to control ($P<0.01$).

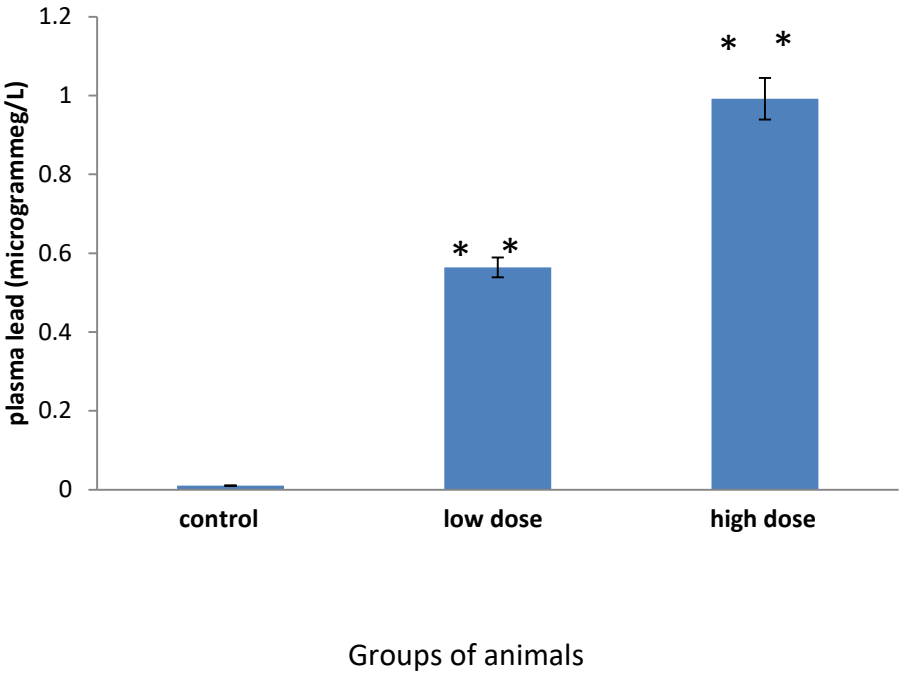


Figure 7: Effects of chronic lead exposure on plasma lead levels in rats

4.3 Macroscopic evaluation of ulcer area after acetic acid induced ulceration in control and lead-treated rats

In table 2 and figure 8 are the effects of chronic lead treatment on ulcer area and percentage change in ulcer area respectively. As healing progressed, on each of days 7, 14 and 21 there were significant differences in the ulcer areas of treated groups compared to control ($P<0.05$). Also there was a significant difference in ulcer areas in each group of animals each day compared to the previous day of measurement ($P<0.05$).

On day 7, the ulcerated area was increased by 221.8% in low dose group and by 319.0% in high dose group compared to control value of 175.6%. On day 14, the ulcerated area was increased by 177.3% and 232.4% for low dose and high dose groups respectively, compared to control value of 107.1%. On day 21, the ulcerated area was increased by 64.9% in low dose group and by 86.2% in high dose group compared to control value of 32.4% ($P<0.05$).

Table 2: Effects of chronic lead treatment on gastric ulcer area (planimetry) after ulcer induction in rats

Groups	**Day 0 (mm^2)**	**Day 7 (mm^2)**	**Day 14 (mm^2)**	**Day 21 (mm^2)**
Control	4.5 ± 0.0	7.90 ± 0.12	4.82 ± 0.14	1.46 ± 0.17
Low dose	4.5 ± 0.0	9.98 ± 0.41*	7.98 ± 0.36 *	2.92 ± 0.11*
High dose	4.5 ± 0.0	14.36 ± 0.21*	10.46 ± 0.32*	3.88 ± 0.34*

*= Significant difference at $P<0.05$

In figure 9a is effects of chronic lead treatment on gastric ulcer healing rate per day after ulcer induction in rats. On day 7, ulcer healing rate was negative in all groups. This indicated that ulcer formation was greater than ulcer healing between days 0 and 7. The value of control group on day 7 was the highest while the treated groups had lower ulcer healing rates of graded levels in a dose-dependent manner. As healing progressed (days 14 and 21), there was increase in ulcer healing rates compared to day 7. On days 14 and 21, there was no significant difference in the ulcer healing rates of treated groups compared to control.

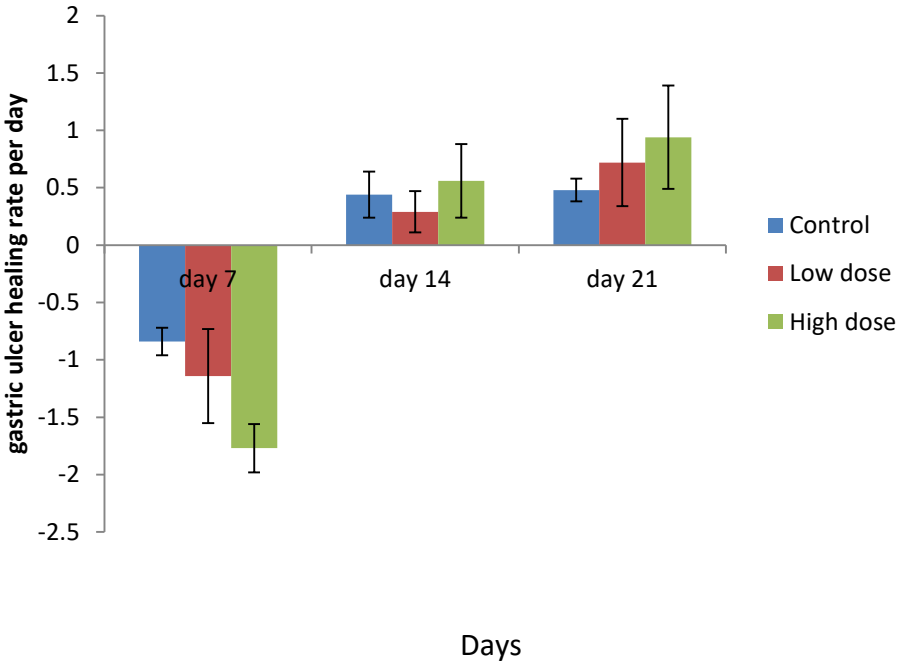


Figure 9a: Effects of chronic Lead exposure on gastric ulcer healing rate per day after ulcer induction in rats

Presented in figure 9b is the effect of chronic lead treatment on total gastric ulcer scores in indomethacin-induced ulcerated rats. In control+indomethacin group, total ulcer score was 12.6. In the low dose+indomethacin group, the total ulcer score was 15.9, an increase of 26.5% while in high dose+indomethacin group, total ulcer score was 24.3, an increase of 92.8% compared to control+indomethacin. The differences of the lead exposed groups compared to control+ indomethacin were significant ($P<0.05$).

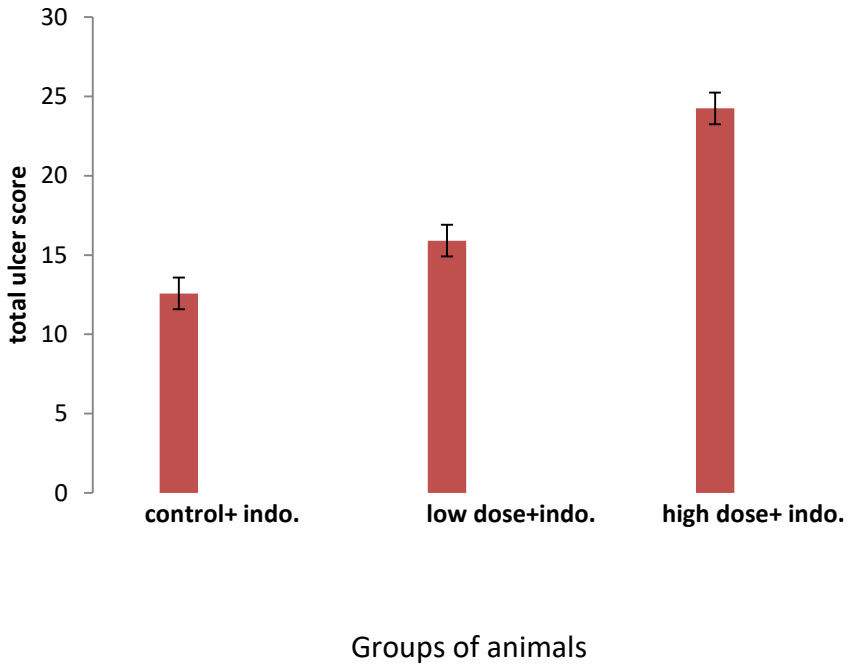


Fig. 9b: Effects of chronic Lead exposure on total ulcer score in indomethacin-induced ulcerated rats

4.4 Microscopic evaluation of slides from the ulcerated area of the stomach in control and lead-exposed rats.

Shown in table 3 is the effects of chronic lead exposure on gastric ulcer width in control and lead treated rats. On day 7, the ulcer width in the low lead (low dose) group was increased by 119.0% of the control value while the ulcer width of the high dose group increased by 109.2% on the same day compared to control value of 100%. On day 14, the ulcer width in the low lead (low dose) group was increased by 129.5 % of the control value while the ulcer width of the high dose group increased by 120.8% on the same day. Finally, on day 21, the ulcer width in the low lead (low dose) group was increased by 134.1 % of the control value while the ulcer width of the high dose group increased by 205.7% on the same day relative to control. All the differences were statistically significant ($P<0.05$).

Table 3: Effects of chronic lead exposure on gastric ulcer width after ulcer induction (histomorphometry)

Groups	Day 0 (µm)	Day 7(µm)	Day 14(µm)	Day 21(µm)
Control	44.20±6.45	550.51±17.77	149.06±3.71	52.84±4.01
Low dose	52.89±2.39	654.93±12.19*	193.20±7.69*	71.05±3.09*
High dose	74.90±7.71*	601.21±38.20*	180.53±14.11*	109.46±9.15*

*= Significant difference at $P<0.05$

Shown in table 4 is the effect of chronic lead exposure on gastric mucosal area eroded in control and lead treated rats. On day 7, the gastric mucosal area eroded in the low dose group was increased by 190.5% of the control value while the ulcer width of the high dose group increased by 208.6% on the same day compared to control value of 100%. On day 14, the mucosal area eroded in the low lead (low dose) group was increased by 196.1% of the control value while the ulcer width of the high dose group increased by 244.3% on the same day. Finally, on day 21, the mucosal area eroded in the low lead (low dose) group was increased by 151.1% of the control value while the ulcer width of the high dose group increased by 417.6% on the same day relative to control. All differences were statistically significant ($P<0.05$).

Table 4: Effects of chronic lead exposure on gastric mucosal area eroded after ulcer induction (histomorphometry)

Groups	Day 0 (μm^2)	Day 7(μm^2)	Day 14(μm^2)	Day 21(μm^2)
Control	$1.02x10^4 \pm 44.04$	$2.58x10^4 \pm 121.38$	$4.61x10^4 \pm 25.34$	$1.31x10^4 \pm 27.4$
Low dose	$1.62x10^4 \pm 16.29$	$4.91x10^4 \pm 83.25$*	$9.04x10^4 \pm 52.54$*	$1.98x10^4 \pm 21.08$
High dose	$3.99x10^4 \pm 52.67$*	$53.81x10^4 \pm 26.09$*	$11.20x10^4 \pm 96.4$*	$5.47x10^4 \pm 62.5$*

*= Significant difference at $P<0.05$

`Presented in figure 10 are the effects of chronic lead exposure on percentage healing rate with respect to day 7 after ulcer induction in control and lead treated rats. On day 7, the percentage healing rate with respect to day 7 was 100% in all groups (reference point). On day 14, the percentage healing rate with respect to day 7 in the low lead (low dose) group was decreased to 81.6 %, while the percentage healing rate with respect to day 7 of the high dose group decreased to 79.1% on the same day compared to the control value of 82.1%. Finally, on day 21, the percentage healing rate with respect to day 7 in the low lead (low dose) group was increased to 96.0 %, while the percentage healing rate with respect to day 7 of the high dose group decreased to 89.8% on the same day relative to control value of 94.9%.

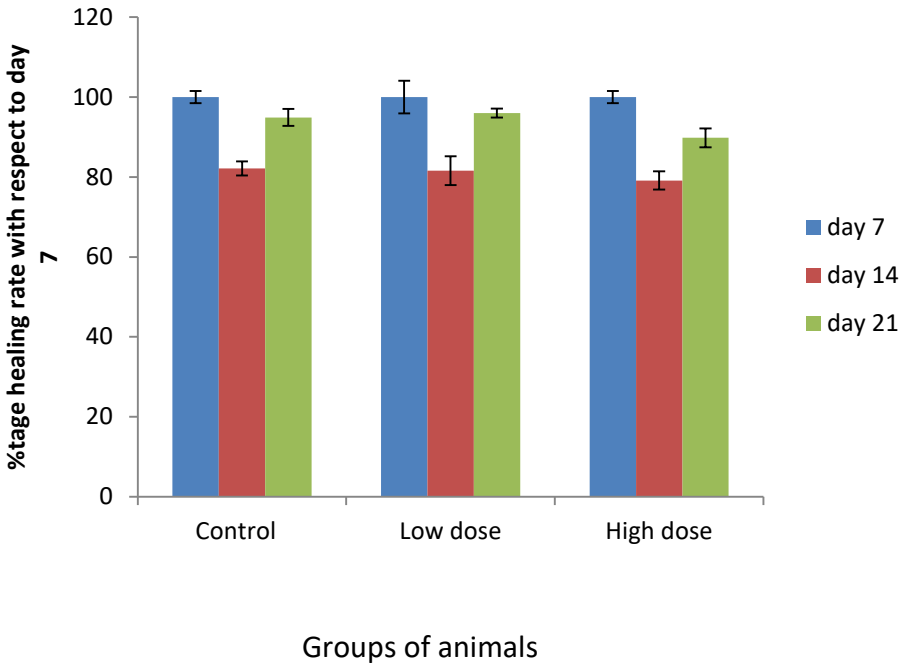


Figure 10: Effects of chronic lead exposure on percentage healing rate with respect to day 7 after ulcer induction in rats (histomorphometry)

4.5-The role of gastric acid in lead-induced delay of gastric ulcer healing

As shown in figure 11, the pH of gastric juice collected from rats in the control group was 1.43. In the low lead group, the pH was 1.43, while the pH in the high lead group was 1.38. When compared with control, the values in the lead-treated groups were not changed significantly.

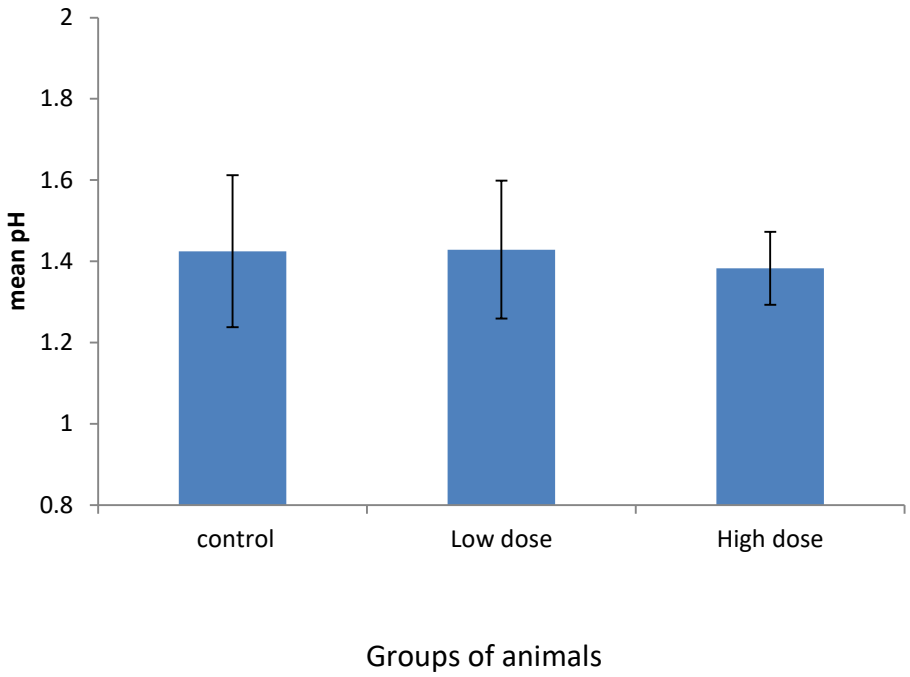


Figure 11: Effects of chronic lead exposure on gastric juice pH in control and lead treated rats

In table 5 and figure 12 show basal gastric acid secretion (BGAS) and percentage changes in control and lead-treated rats after acetic acid induced ulceration. On day 0 (baseline), there was no significant difference between BGAS of the treatment groups and control. As healing progressed (days 7-21) the BGAS decreased progressively in all groups. In the control group, BGAS on day 0 was 2.38±0.34 mEq/L. This was decreased to 1.48± 0.17 (62.2%), to 1.24 ± 0.28 (52.1%) and 0.94±0.07 (39.5%) on days 7, 14 and 21 respectively. In the low lead treated animals, BGAS was 2.5± 0.38 mEq/L on the day of ulcer induction. However, BGAS decreased to 1.84± 0.26 (73.6%), and to1.38± 0.25 (55.2%) and 1.50±0.22 (60%) on days 7, 14 and 21 respectively. In the high lead treated group, BGAS on the day of ulcer induction was 2.50± 0.41mEq/L. This was decreased to 2.16 ± 0.34 (86.4%) on day 7, 1.96 ± 0.31 (78.4%) on day 14 and 1.74±0.17 (69.6%) on day 21 respectively. It was noted that at the end of the experiment, control animals had the lowest absolute BGAS and the difference was significant when compared to high dose group.

Table 5: Basal gastric acid output in control and lead-treated rats after acetic acid induced ulceration

Groups	Day 0 (mEq/L x1000)	Day7(mEq/L x1000)	Day 14 (mEq/L x1000)	Day21(mEq/L x 1000)
Control	2.38± 0.34	1.48 ± 0.17	1.24 ± 0.28	0.94±0.07
Low dose	2.50± 0.38 ^{NS}	1.84 ± 0.26	1.38± 0.25 ^{NS}	1.50±0.22*
High dose	2.50± 0.41 ^{NS}	2.16 ± 0.34*	1.96 ± 0.31 *	1.74±0.17 *

NS= Not significant.

*= Significant difference at $P<0.05$.

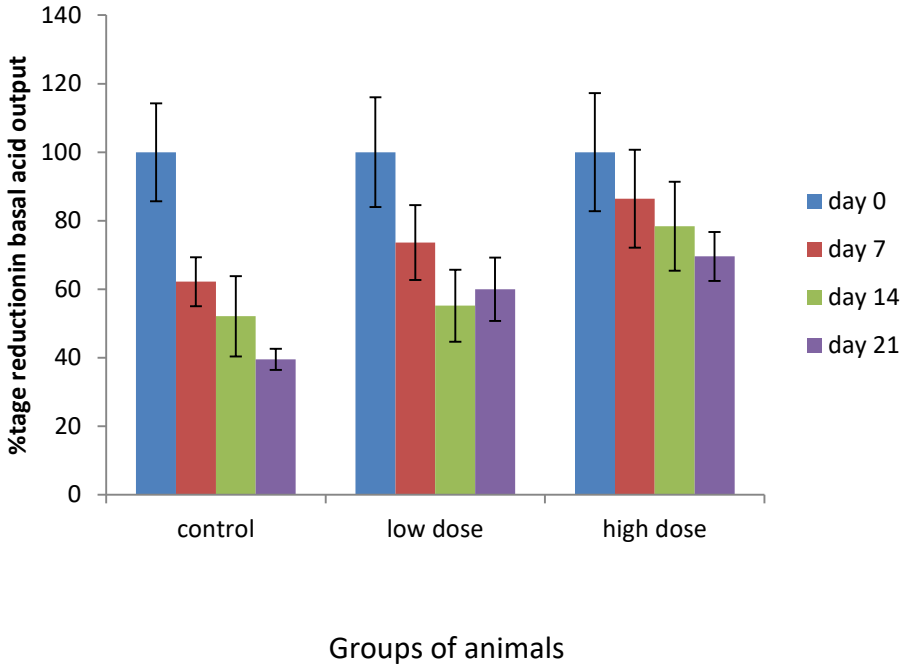


Figure 12: Percentage reduction in basal gastric acid secretion in control and lead treated rats after ulcer induction.

Shown in table 6 and figure 13 are peak histamine-stimulated gastric acid (PHSGA) output and the percentage changes in PHSGA of control and lead-treated rats after acetic acid induced ulceration. On day 0, there was no significant difference between the PHSGA of the control and treatment groups ($P<0.05$).

As healing progressed (days 7-21), PHSGA decreased progressively in all groups. In the control group, PHSGA secretion on day 0 was 5.18±0.41mEq/L. This was decreased to 4.24±0.20 mEq/L (81.9%), and to 3.52±0.37mEq/L (68.0%) and 2.50±0.12mEq/L (48.3%) on days 7, 14 and 21 respectively. In the low lead treated animals, PHSGA secretion was 5.17±0.46 mEq/L on the day of ulcer induction. However, PHSGA secretion decreased to 4.22±0.34 mEq/L (81.6 %), and to 3.58±0.53 (69.2 %) and 3.70±0.48 (71.6 %) on days 7, 14 and 21 respectively. In the high lead treated group, PHSGA output on the day of ulcer induction was 5.22±0.33 mEq/L. This was decreased to 4.84±0.39 mEq/L (92.7%) on day 7, 4.44±0.23 mEq/L (85.1%) on day 14 and 3.92±0.28mEq/L (75.1%) on day 21 respectively. Note that at the end of the experiment, control animals had the lowest absolute PHSGA output and the difference was significant when compared to both low and high dose groups.

Table 6: Effects of chronic lead exposure on peak histamine-stimulated gastric acid secretion after acetic acid induced ulceration in rats

Groups	Day 0 (mEq/L X1000)	Day 7 (mEq/L X1000)	Day 14 (mEq/L X1000)	Day 21(mEq/L X1000)
Control	5.18±0.41	4.24±0.20	3.52±0.37	2.50±0.12
Low dose	5.17±0.46 ^{NS}	4.22±0.34^{NS}	3.58±0.53^{NS}	3.70±0.48*
High dose	5.22±0.33 ^{NS}	4.84±0.39^{NS}	4.44±0.23^{NS}	3.92±0.28*

NS= Not significant.

*= Significant difference at $P<0.05$.

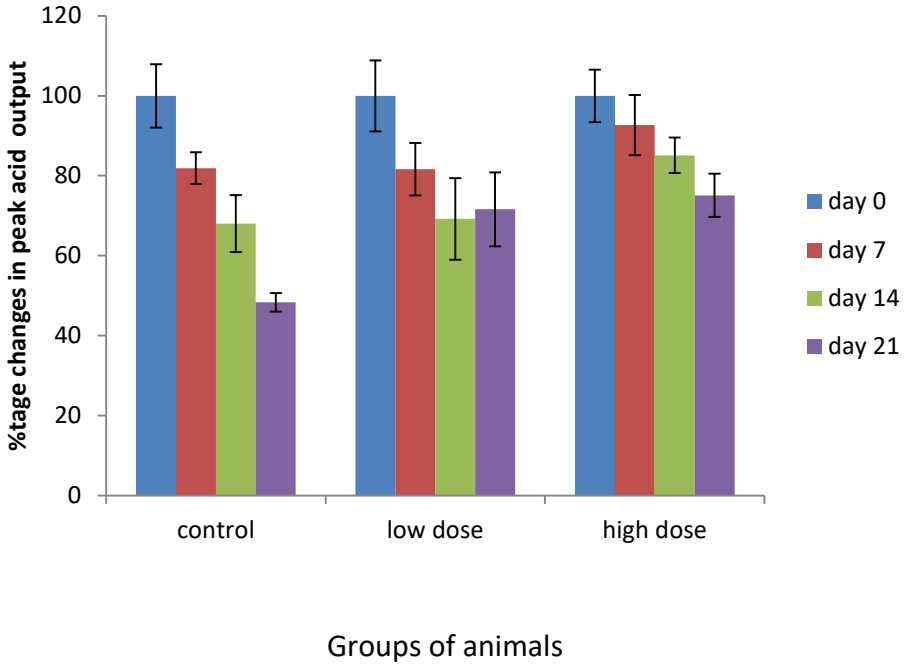


Figure 13: Percentage changes (reduction) in peak Histamine-stimulated gastric acid secretion in control and lead treated rats after acetic acid-induced ulceration

Presented in Table 7 are effects of chronic lead exposure on both basal and peak histamine stimulated gastric acid secretion after ulcer induction with indomethacin.

There was a significant increase ($P<0.05$) in the BGAS of group 6 when compared with groups 1, 2 and 4. There was no significant increase ($P<0.05$) in BGAS of group 6 when compared with groups 3 and 5. There was also significant increase ($P<0.05$) in the PHSGA output of group 6 compared with groups 1,2,3,4 and 5.

Table 7: Effects of chronic lead exposure on basal and Peak histamine stimulated Gastric acid secretion in indomethacin-induced gastric ulcerated animals.

	Groups	**Basal acid secretion**	**Peak acid output**
1	Control	0.58 ± 0.16	1.34±0.21
2	Low dose lead	0.68±0.05	1.3±0.04
3	High dose lead	0.78±0.05	1.36±0.07
4	Control + indomethacin	0.48 ± 0.06	1.3±0.27
5	Low dose lead + indomethacin	0.85 ± 0.05*	1.6±0.12
6	High dose lead + indomethacin	0.98 ± 0.08*	1.85±0.09*

*= significant difference at $P<0.05$

As shown in Figure 14, the parietal cell count (PCC) obtained from gastric tissue of rats in the control group was 102 cells/ unit area. In the low lead group, the PCC was 108 cells per unit area, while the PCC in the high lead group was 118 cells per unit area. When compared with control, the values in the high lead group was significantly different while in the low lead group, it was not ($P<0.05$).

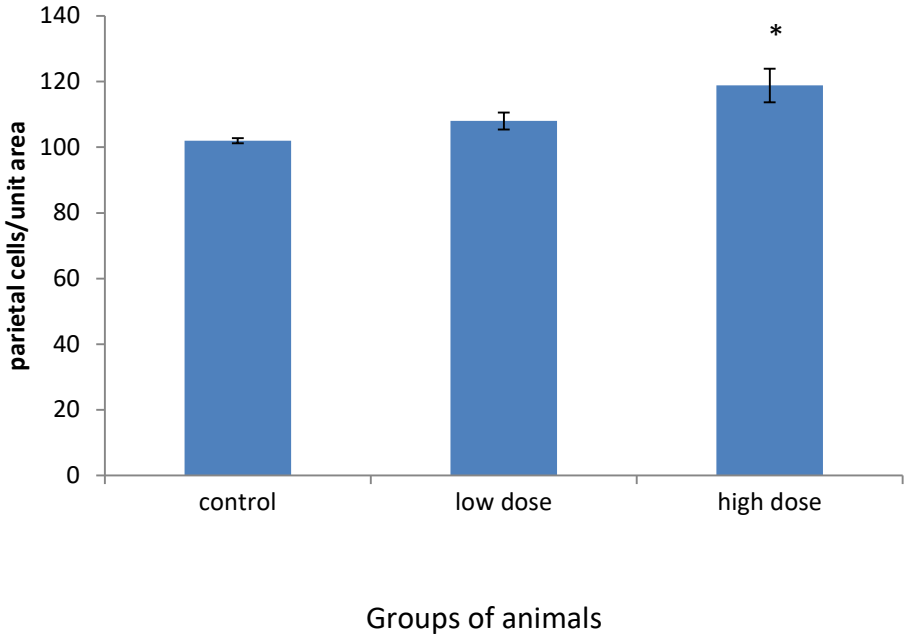


Figure 14: Effects of chronic lead exposure on gastric parietal cell count in control and lead treated rats

4.6-Changes seen in goblet cell count in acetic acid induced ulcerated control and lead treated rats

As shown in figure 9, on day zero, goblet cells count in both treated groups were 25.4 and 22.2 cells/unit area (for low and high doses respectively) compared to control value of 57.0 cells/unit area. The differences were significant ($P<0.01$). In the control animals during healing, goblet cell count increased from 44.2 on day 7 to 55.2 on day 14, but by day 21, it reduced to 34.2 cells /unit area.

During healing (days 7-21), in both treated groups, there were insignificant changes in goblet cell counts as healing progressed (days 7-21). The goblet cell counts were significantly lower in the treatment groups compared to control ($P<0.05$) during healing except in day 21 which was not significant.

Table 9: Effects of 20 weeks lead exposure on goblet cell count after ulcer induction in rats

	Day 0 (cells/unit area)	**Day 7 (cells/unit area)**	**Day 14 (cells/unit area)**	**Day 21 (cells/unit area)**
Control	57±1.64	44.2±2.03	55.2±1.77	34.2±1.65
Low dose	25.4±1.21*	32.8±0.86*	33.2±0.86*	31±0.45 ^{NS}
High dose	22.2±0.86*	33.8±1.16*	32.2±1.02*	35.6±1.21 ^{NS}

NS=Not significant.

*= Significant difference ($P<0.05$).

4.7-Electrolyte changes in the blood of rats during ulcer healing in control and lead exposed rats

As shown in Figure 15, there was no significant difference in the plasma sodium ion concentrations across all groups and days ($P<0.05$). In the control group, sodium ion concentration was 142.2mmol/L on day 0. By day 21, the value was 140.2mmol/L. in the low dose group, sodium ion concentration on day 0 was 143.0mmol/L and by day 21, the value was 143.2mmol/L while in the high dose group, day 0 value was 143.2 and day 21 value was 133.8mmol/L.

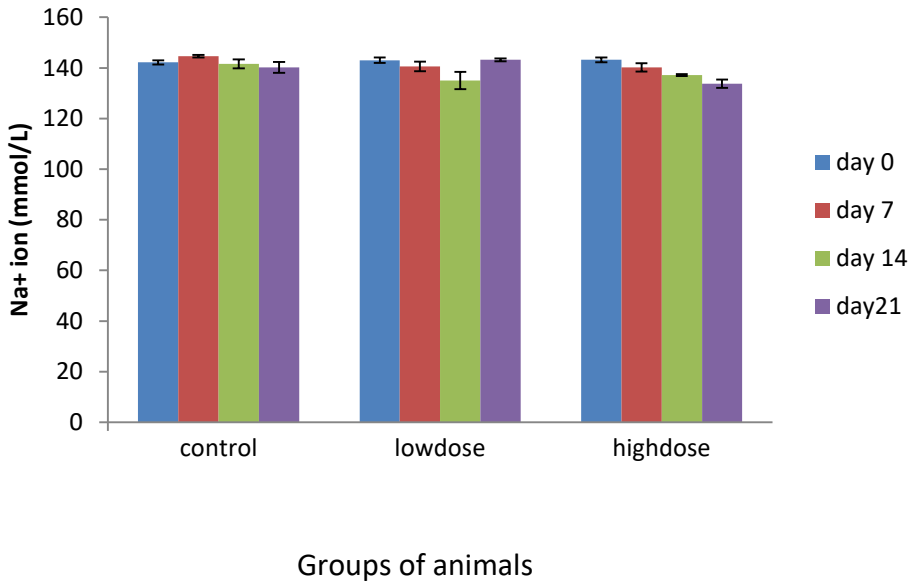


Figure 15: Effect of chronic lead exposure on plasma sodium ion (Na^+) concentrations after ulcer induction in rats

As shown in Figure 16, on day 0, urea levels of the treatment groups were significantly higher than the control ($P<0.05$). The value for low dose group was 46.4 mg/dL while the value for high dose group was 31.8 mg/dL compared to the control value of 25.0 mg/dL. During days 7-21, there were insignificant changes in plasma urea levels between the groups and across days. In control group, the values for plasma urea concentration were 36.2, 36.8 and 34.6 mg/dL for days 7, 14 and 21 respectively. In the high dose group, the corresponding values for plasma urea concentration were 34.2, 32.2 and 32.6 mg/dL.

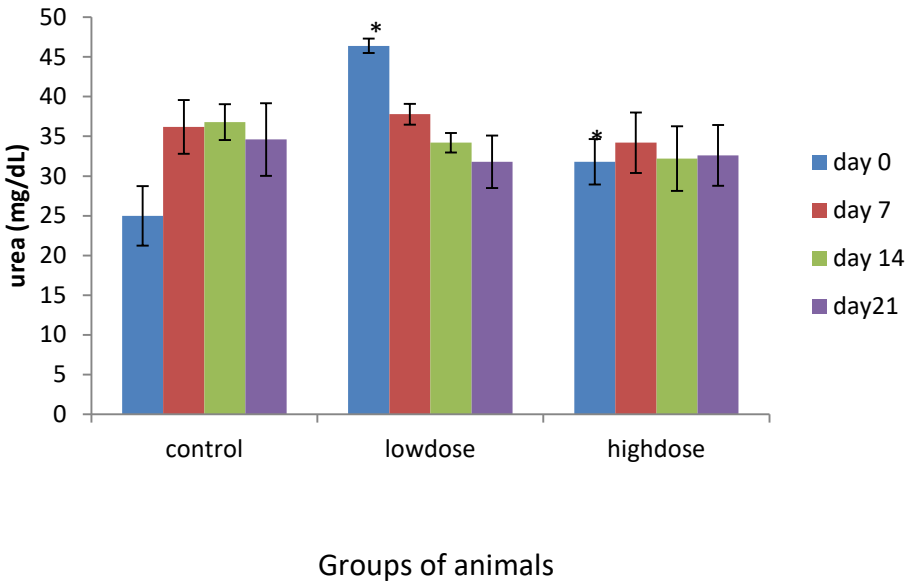


Figure 16: Effects of chronic lead exposure on plasma urea concentrations after ulcer induction in rats

As shown in Figure 17, there were variations in the plasma calcium ion concentration across all groups. On day 0, calcium levels of the treatment groups were 127.3ppm and 151.0ppm compared to the control value of 138.1ppm. During days 7-21, there were insignificant changes in plasma calcium levels between the groups and across days. In control group, the values for calcium concentrations were 112.7, 147.9 and 104.3ppm for days 7, 14 and 21 respectively. In the high dose group, the values for calcium ion concentrations were 141.1, 138.8 and 133.3ppm for days 7, 14 and 21 respectively.

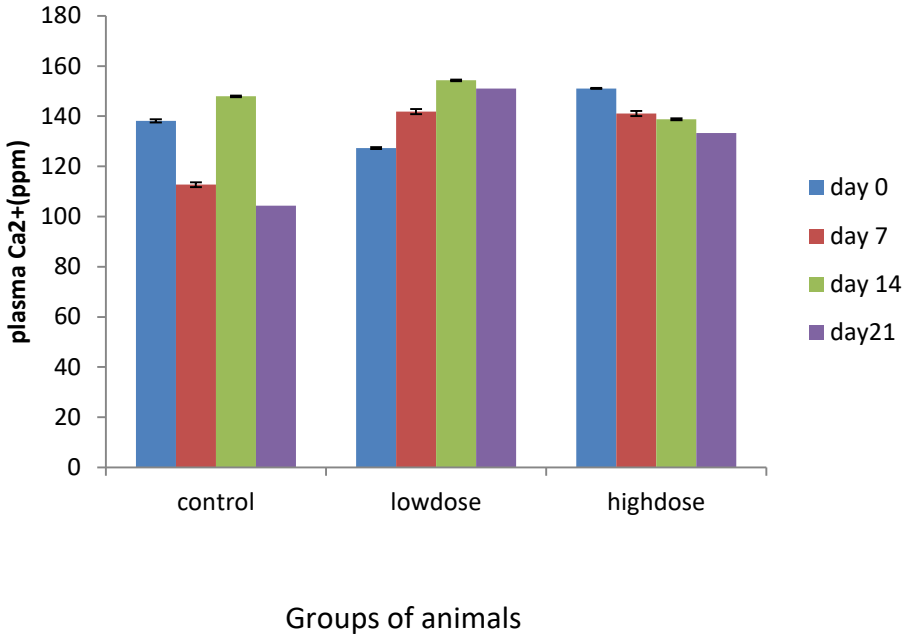


Figure 17: Effects of chronic lead exposure on plasma calcium ion (Ca^{2+}) concentration after ulcer induction in rats.

As shown in Figure 18, there were no significant differences in the Chloride ion concentrations between control and treated groups except high dose (day 0) ($P<0.05$). On day 0, plasma chloride levels of the treatment groups were 103.4 mmol/L and 138.6 mmol/L compared to the control value of 105.6 mmol/L. During days 7-21, there were insignificant changes in plasma chloride levels between the groups and across days. In the control group, the values for chloride were 103.6, 102.2 and 100.8 mmol/L for days 7, 14 and 21 respectively. In the high dose group, the values for chloride ion concentrations were 96.8, 98.8 and 95.2mmol/L for days 7, 14 and 21 respectively.

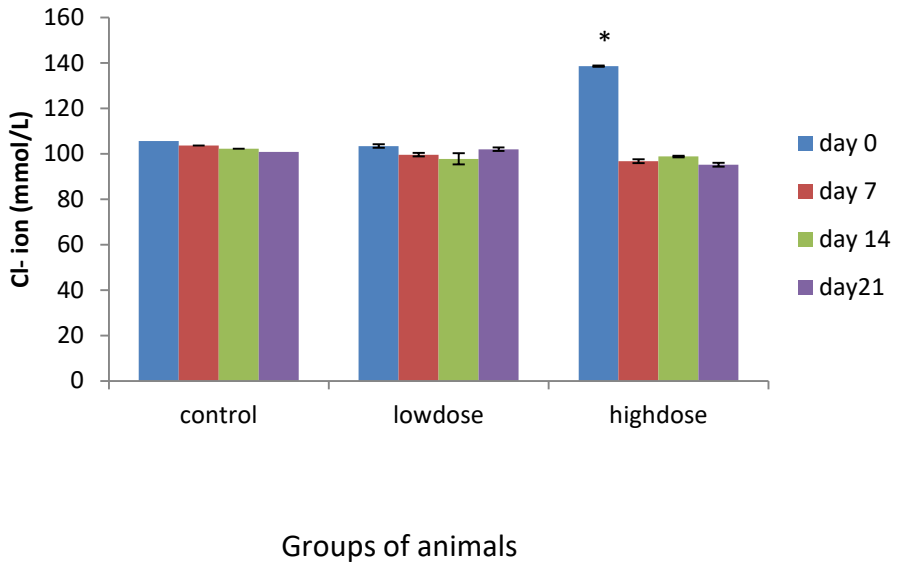


Figure 18: Effect of chronic Lead exposure on plasma chloride ion (Cl^-) concentration after ulcer induction in rats

As shown in Figure 19, there were no significant differences in the plasma bicarbonate ion concentration between control and treated groups. On day 0, plasma bicarbonate levels of the treated groups were 20.8 mmol/L and 21.8 mmol/L compared to the control value of 19.0 mmol/L. During days 7-21, there were insignificant changes in plasma bicarbonate levels between the groups and across days. In control group, the values for bicarbonate ion concentrations were 20.4, 22.8 and 18.2 mmol/L for days 7, 14 and 21 respectively. In the high dose group, the values for bicarbonate ion concentrations were 20.6, 19.6 and 20.8mmol/L for days 7, 14 and 21 respectively.

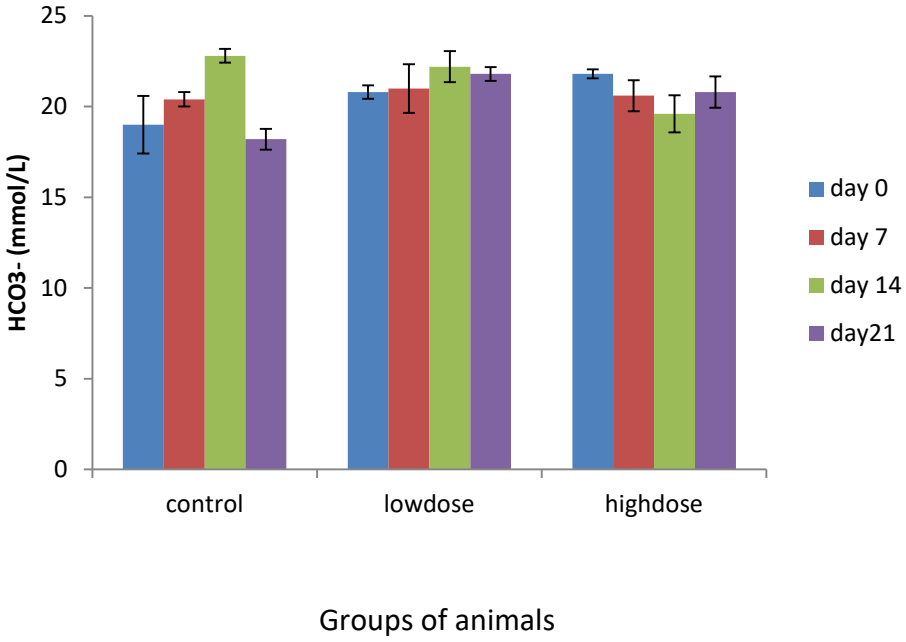


Figure 19: Effects of chronic lead exposure on plasma bicarbonate ion concentrations after ulcer Induction in rats

As shown in Figure 20, there were no significant changes between the control and low dose groups. On day 0, plasma K^+ levels of the high dose group was 12.5mmol/L compared to low dose and control values of 5.0 and 5.1 mmol/L respectively. During days 7-21, there were insignificant changes in plasma K^+ levels within each group except in the high dose group. In the control group, the values for K+ ion were 4.7, 5.2. and 4.9 mmol/L for days 7, 14 and 21 respectively. In the high dose group, the values for K+ ion concentrations were 10.7, 9.2 and 11.2mmol/L for days 7, 14 and 21 respectively.

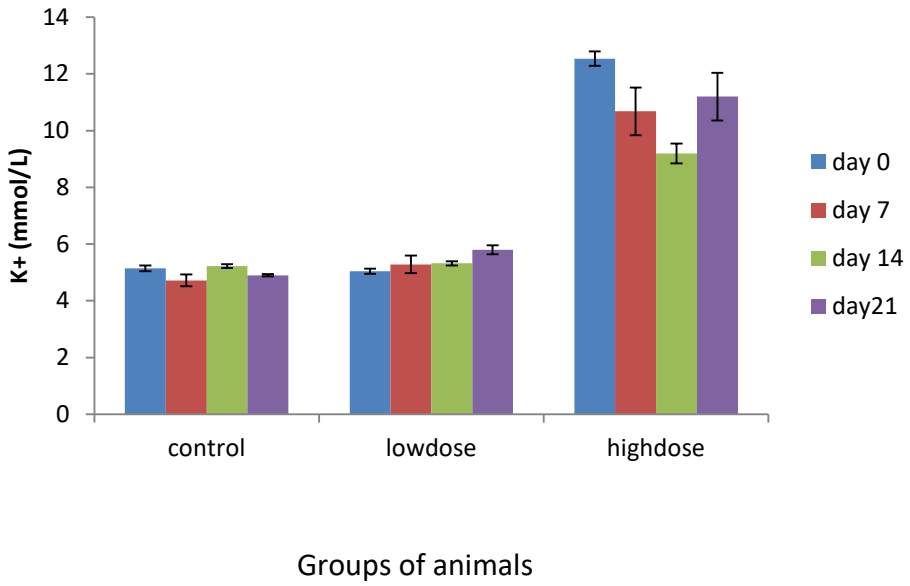


Figure 20: Effects of chronic lead exposure on plasma potassium ion (K^+) concentration after ulcer induction in rats

4.8- Changes in hematological profile of rats during ulcer healing in control and lead-exposed rats

As shown in Figure 21, on day 0 there were no significant differences in the red blood cell counts between the control and treatment groups ($P<0.05$). The values were $4.97x10^{12}$, $4.56x10^{12}$ and $4.28x10^{12}$ respectively for control, low dose and high dose groups. As healing progressed (days 7-21) there were significant increases in the red blood cell counts in control group only ($5.38\ x10^{12}$ $6.96x10^{12}$ and $7.47x10^{12}$ for days 7, 14 and 21 respectively) while in the low dose and high dose groups, there were no significant changes.

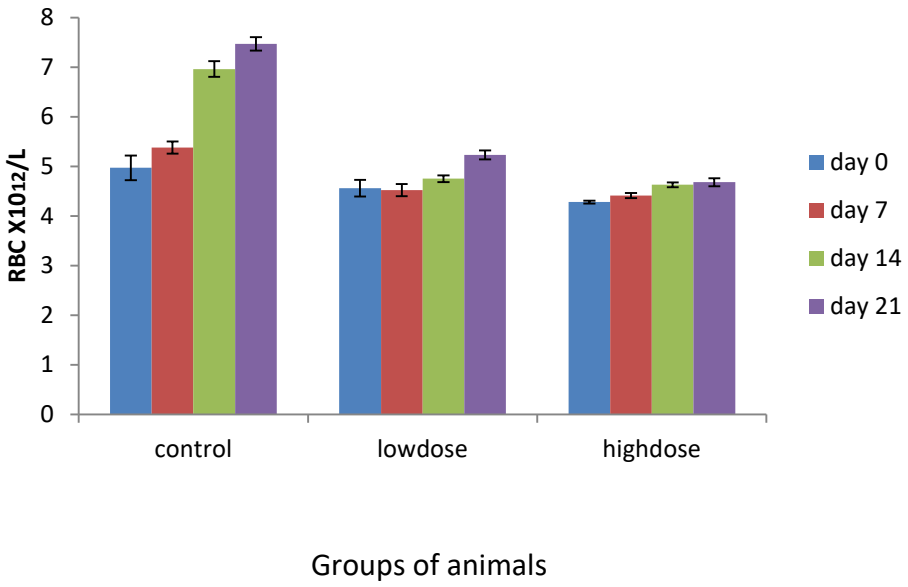


Figure 21: Effects of chronic lead exposure on red blood cell count after ulcer induction in rats

As shown in Figure 22, on day 0 the values for packed cell volume (PCV) were 40.4%, 36.2% and 29.6% for control, low dose and high dose groups respectively. There was a significant difference in the PCV between control and the high dose group, while the difference between control and the low dose group was insignificant ($P<0.05$). In all groups, there was gradual increase in PCV as healing progressed . In the control group during healing, the values were 40.8%, 46.6% and 48.6% for days 7, 14 and 21 respectively. In the high dose group, the PCV was to 31.4%, 33.6% and 35.8% respectively for days 7, 14 and 21.

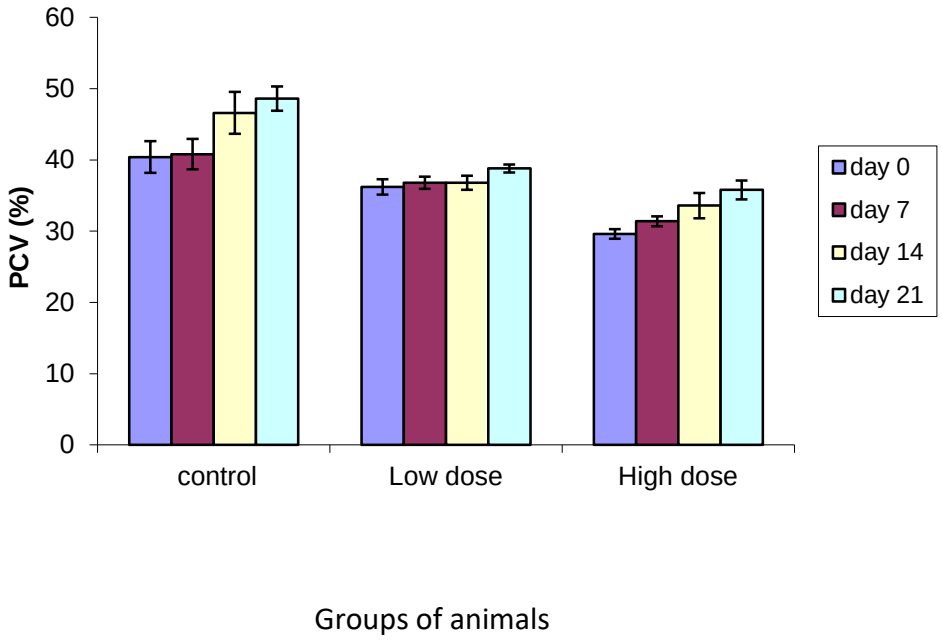


Figure 22: Effects of chronic lead exposure on packed cell volume after ulcer induction in rats.

Presented in Figure 23 are the results of chronic lead exposure on white blood cell (WBC) count after ulcer induction in rats.. The result showed that there was no significant difference between the WBC count of control and high dose group on day 0. However, there was a significant reduction in low dose group compared to control on day 0. On day 0 the values for WBC counts were $8510x10^6$, $5700x10^6$ and $8780x10^6$ for control, low dose and high dose groups respectively. Between days 7, 14 and 21, control values for WBC count were $8530x10^6$, $8860x10^6$ and 8050 $x10^6$. The WBC count values for high dose was $8240x10^6$, $8620x10^6$ and $9840x10^6$ respectively for days 7, 14 and 21. These values were not significantly different from the corresponding control values except on day 21.

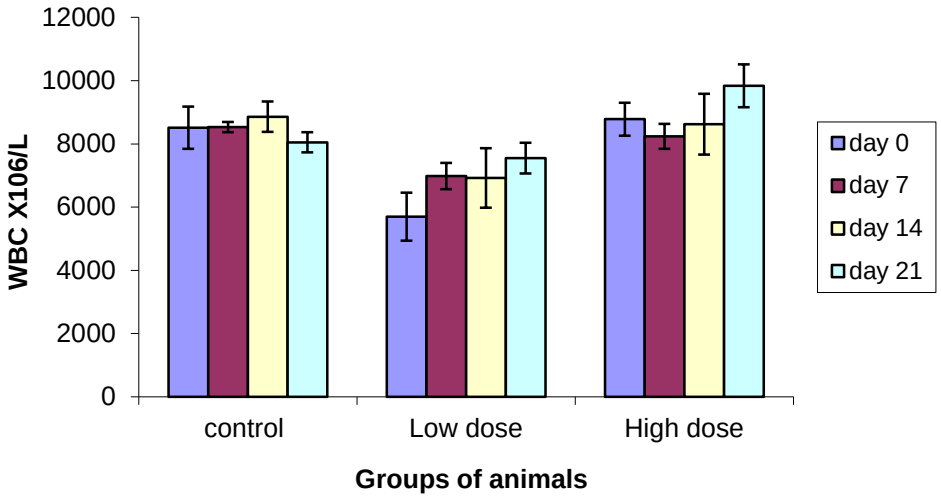


Figure 23: Effects of chronic lead exposure on white blood cell count after ulcer induction in rats

Presented in Figure 24 are the results of chronic lead exposure on platelet count after ulcer induction in rats. Throughout the study, there were no significant differences in the platelet counts between control and the treated groups ($P<0.05$).). On day 0 the values for platelet counts were 114400 platelets/liter, 116200 and 110400 platelets/liter for control, low dose and high dose groups respectively. Between days 7, 14 and 21, control values for platelet counts were 129200, 123400 and 111200 platelets/liter. The platelet count values for high dose were 114600, 116400and 105000 respectively for days 7, 14 and 21. These values were not significantly different from the corresponding control values .

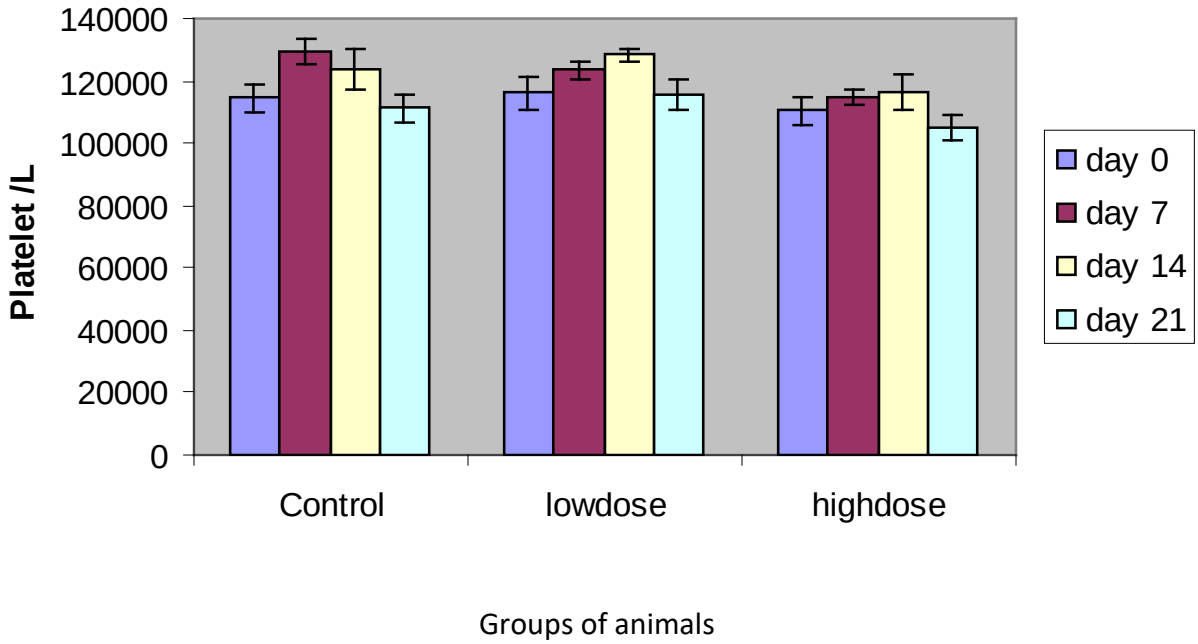


Figure 24: Effects of chronic lead exposure on platelets count after ulcer induction in rats.

Presented in Figure 25 are effects of chronic lead exposure on neutrophil count as a percentage of total white blood cell (WBC) count. This result shows that chronic lead exposure causes progressive increase in neutrophil count as a percentage of total WBC count in rats. The value for control was 36.4%, for low dose the value was 47.8% and for high dose, it was 53.0%. There was significant difference between the control and high dose groups only.

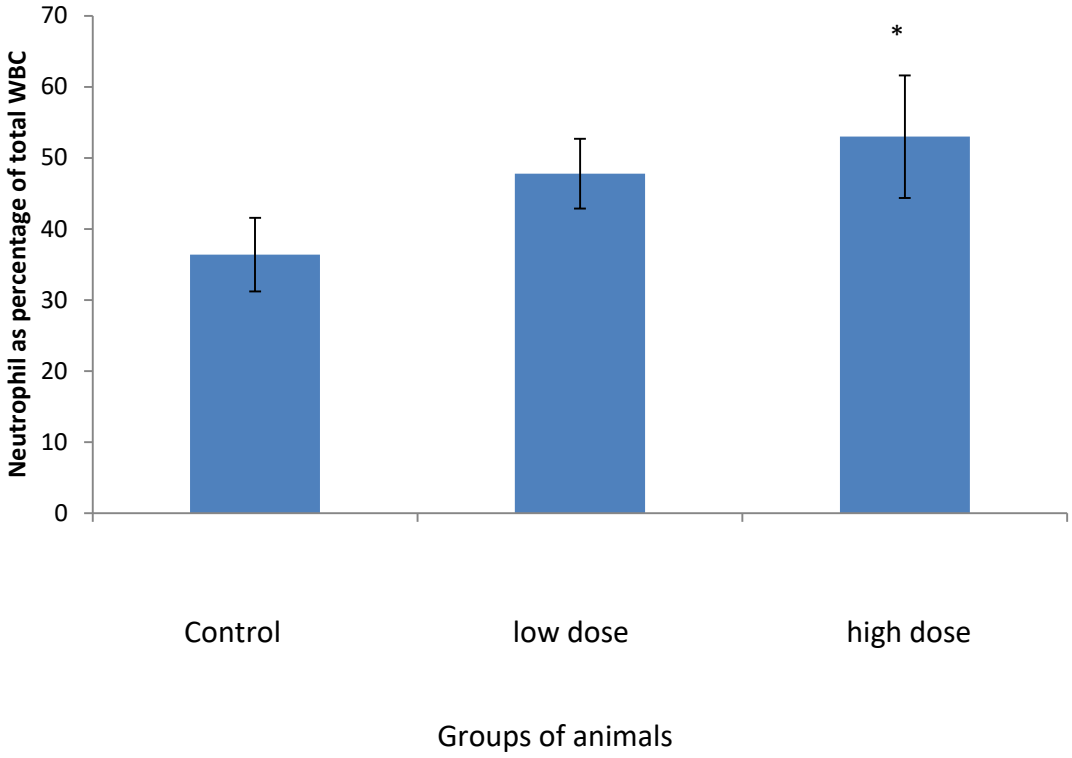


Figure 25: Effect of chronic lead exposure on neutrophil count as a percentage of total white blood cell count in rats

As shown in Table 10, on day 0, there were significant differences in the NLR of control animals compared to the treatment groups ($P<0.05$). The values were 0.77,0.52 and 0.37 for control, low dose and high dose groups respectively. As healing progressed on days 7-21, there were insignificant changes in the NLR of control group (from 0.54 to 0.47 and 0.53). In the low dose group, the values were 0.48, 0.40 and 0.49. When compared to control these values were not statistically different. In the high dose group, the values were 0.32, 0.36 and 0.38. Each of these values were significantly different from the corresponding control values ($*P<0.05$).

Table 10: Effect of chronic lead exposure on neutrophil/ lymphocyte ratio after ulcer induction in rats

	NLR (Day 0)	NLR (Day 7)	NLR (Day 14)	NLR (Day 21)
Control	0.77	0.54	0.47	0.53
Low dose	0.52*	0.48 NS	0.40 NS	0.49 NS
High dose	0.37*	0.32 *	0.36 *	0.38 *

NS= not significant

NLR= neutrophil/lymphocyte ratio

*= significant difference

4.9-The role of oxidative stress in delayed healing of gastric ulceration in control and lead exposed rats

Presented in Figure 26 are effects of chronic lead exposure on gastric tissue lipid peroxidation after ulcer induction in rats. On day 0, gastric malondialdehyde (MDA) activities in both treated groups were higher than control, the difference was significant in high dose only ($P<0.05$). The values were 4.9, 5.8 and 6.7 micromol MDA/g of wet tissue. During healing, MDA Activities in all groups peaked on day 7, (values were 12.5, 13.2 and 40.5 for control, low dose and high dose respectively). By day 21, these values had decreased to 5.4, 7.9 and 20.5 for control, low dose and high dose groups respectively.

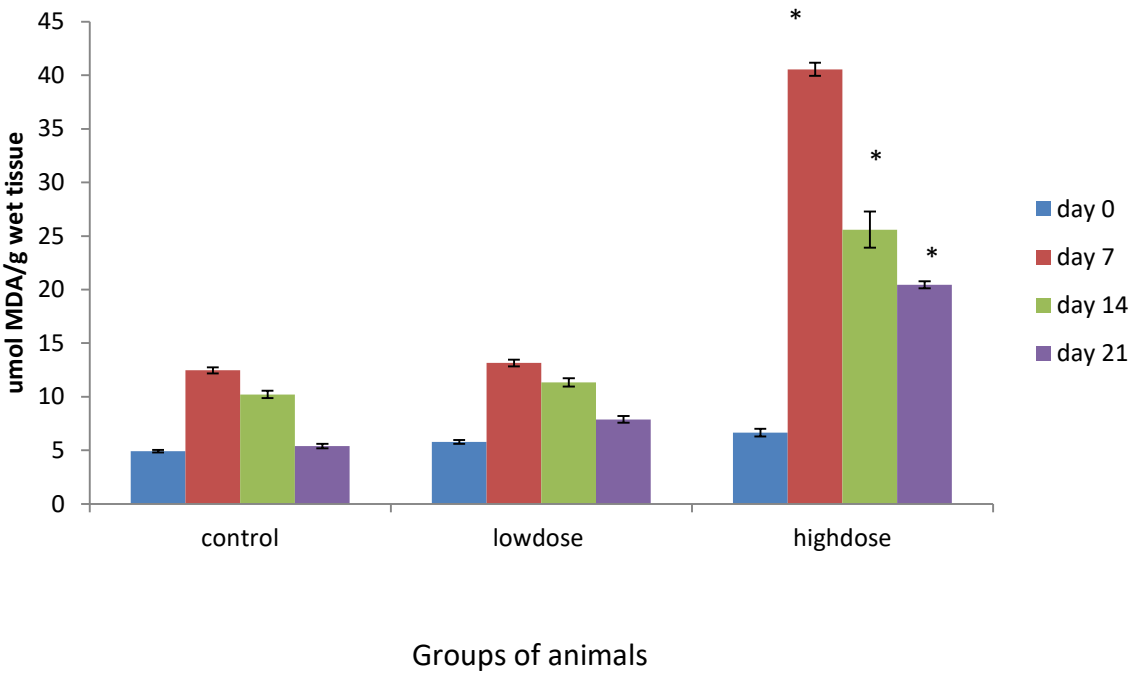


Figure 26: Effects of chronic lead exposure on gastric tissue lipid peroxidation after ulcer induction in rats

Presented in Table 11 are effects of chronic lead exposure on gastric tissue superoxide dismutase (SOD) activity after ulcer induction in rats On day 0, gastric SOD activity in treated groups were higher than in control, the values were 1.5, 1.9 and 2.0 microgramme/mg protein for control, low dose and high dose respectively. The differences between control and treated groups were significant ($P<0.05$). During healing, there were no significant difference in SOD activities in all groups compared to control.

Table 11: Effects of chronic lead exposure on gastric superoxide dismutase activity after ulcer induction in rats

Groups	Day 0 (µg/mg protein)	Day 7(µg/mg protein)	Day 14(µg/mg protein)	Day 21(µg/mg protein)
Control	1.51±0.022	1.83±.009	1.83±0.0027	1.83±0.006
Low dose	1.94±0.004*	1.82 ± 0.011^{NS}	1.77 ± 0.011^{NS}	1.83 ± 0.022^{NS}
High dose	1.96±0.005*	1.88 ± 0.005^{NS}	1.92 ± 0.003^{NS}	1.92 ± 0.006^{NS}

NS = Not significant

* significant compared to control ($P<0.05$).

Presented in Figure 27 are effects of chronic lead exposure on gastric tissue catalase activity after ulcer induction in rats. There were no significant differences in catalase activities throughout the duration of the study. On day 0, the values were 4.30, 4.25 and 4.29 for control, low dose and high dose groups respectively. On day 21.the values were 4.30, 4.32 and 4.27 for control, low dose and high dose respectively.

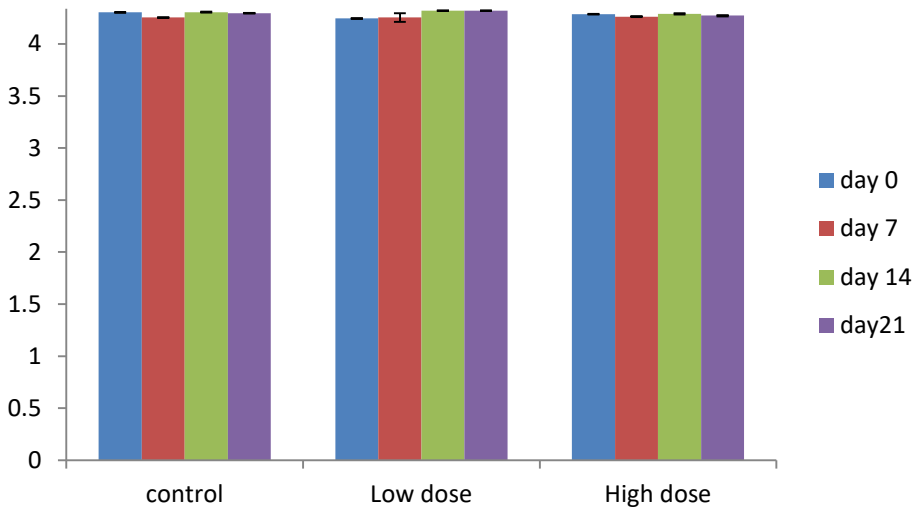


Figure 27: Effects of chronic lead exposure on gastric catalase activity after ulcer induction in rats

4.10- The role of apoptotic cell damage on delayed ulcer healing induced by lead

Presented in Figure 28 are effects of chronic lead exposure on gastric mucosal cell apoptosis after ulcer induction in rats.

During gastric ulcer healing in male albino rats, on days 7, 14 and 21, apoptosis was significantly higher in lead treated animals compared to control ($P<0.05$). The values were 3.9, 9.8 and 2.8 apoptotic cells/unit area in the control group. In the high dose group, apoptosis increased to 25.3, 23.8 and 19.3 apoptotic cells/unit area.

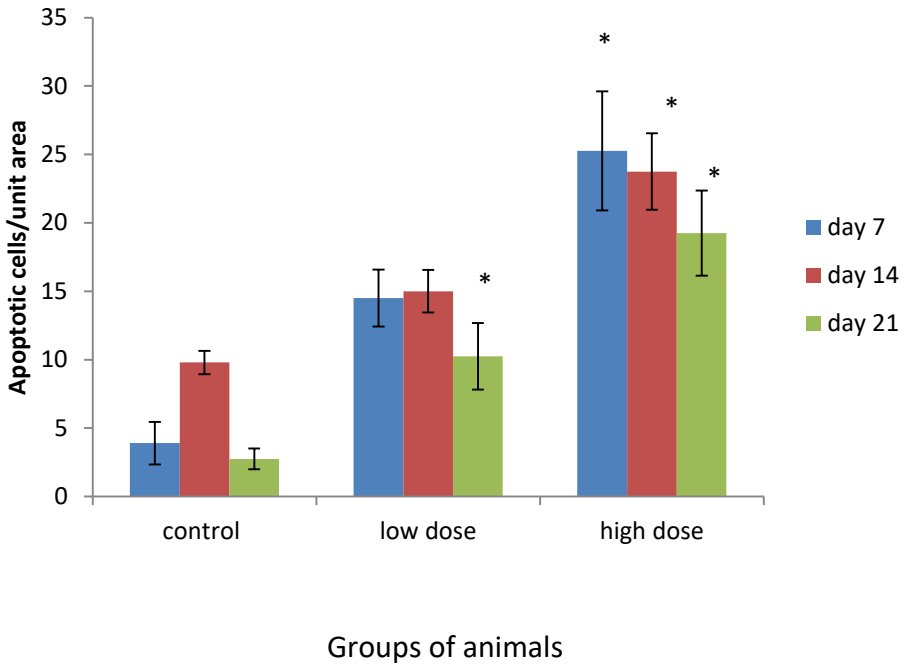


Figure 28: Effects of chronic lead exposure on gastric mucosal cell apoptosis after ulcer induction in rats

4.11 Histological observations of rat stomach during ulcer healing in control and lead-exposed rats

Presented in Plate 2 are gastric mucosal photomicrographs from control, low dose and high dose rats. It shows that there was normal distribution of cells, the tissues were intact, however submucosa fibrosis (black arrow) was seen in the control animal. H&E X100.

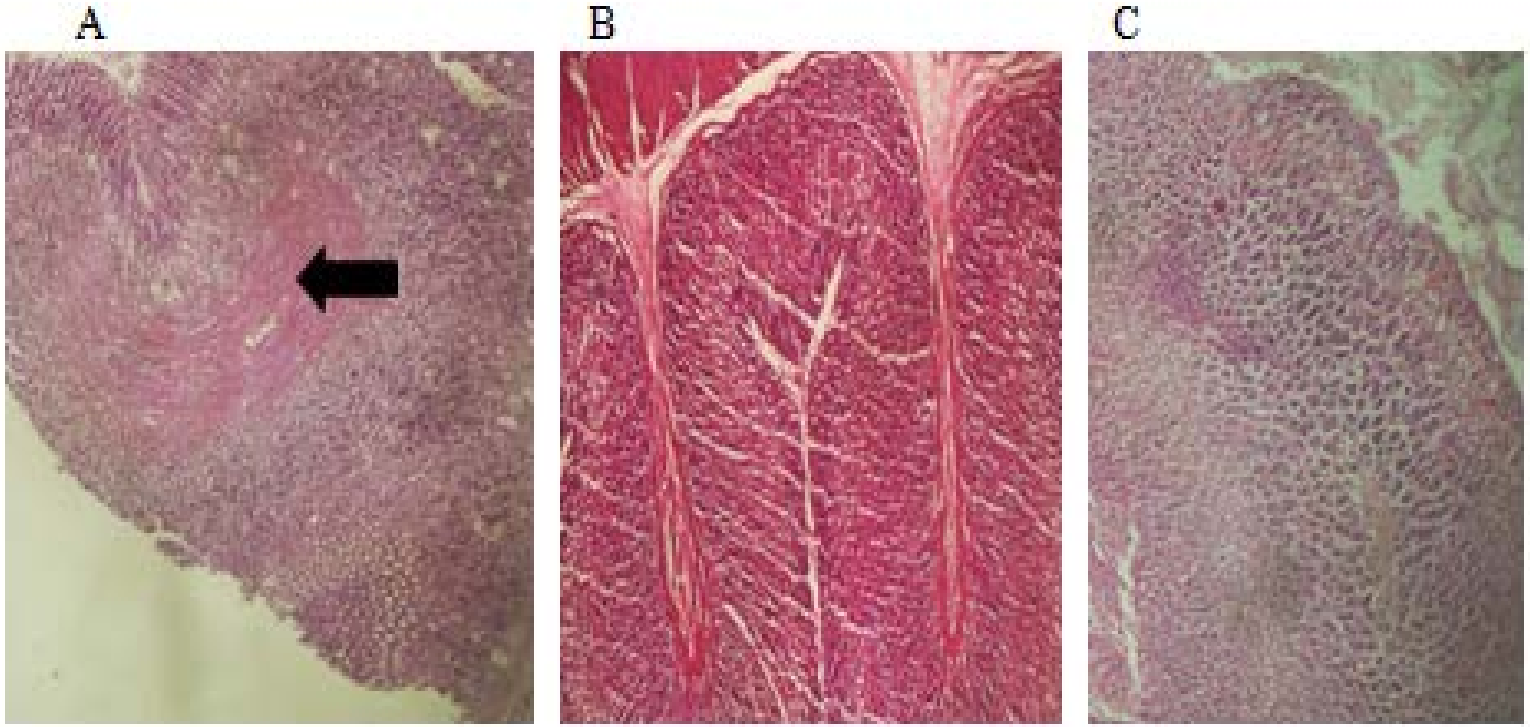


Plate 2: Photomicrographs showing gastric mucosal sections from (A) control, (B) low dose and (C) high dose rats (day 0)

Presented in Plate 3 are gastric mucosal photomicrographs from control, low dose and high dose rats obtained on day 7.There was **i**n (A) focal area of intracellular mucin production (blue arrow) PASX100. In (B) there was focal area of chronic mucosa ulcer (white arrow) with submucosa edema (black arrow) and epithelial mucin production (yellow arrow). H&E X100. And in (C) granulation tissue formation (red arrows) was seen H&E X 100.

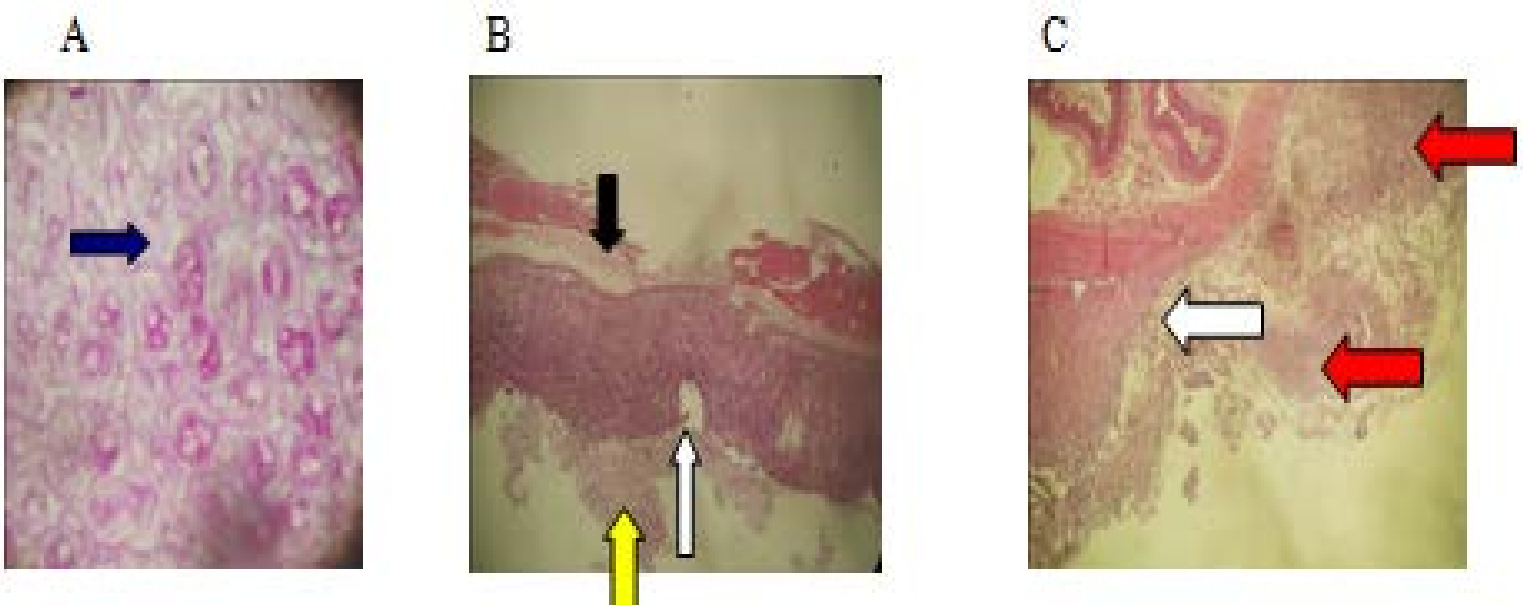


Plate 3: Photomicrographs showing gastric mucosal sections from (A) control, (B) low dose and (C) high dose animals (day 7)

Presented in Plate 4 are photomicrographs of gastric mucosa from low dose rats obtained on day 7. There was (A) hypertrophied muscle layer (black arrow) with focal ulceration (white arrows), abundant neutrophils (yellow arrows) and muscle degeneration (M.D) H&E X100. In (B) i observed focal area of surface epithelial mucin production and intracellular mucin production (blue arrows) PAS X100.

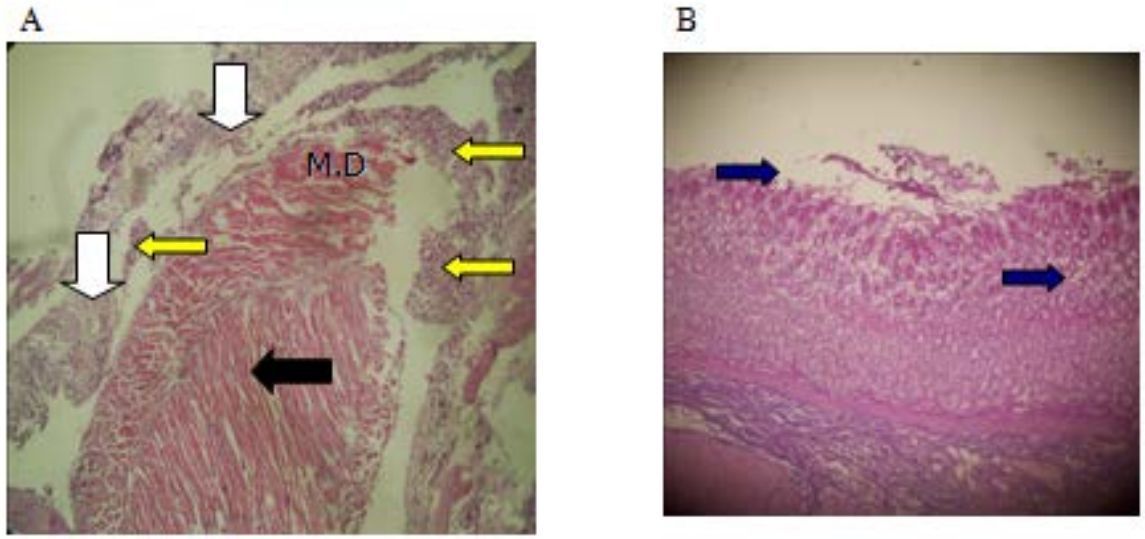


Plate 4: Photomicrographs showing gastric mucosal sections from low dose rats (day 7)

Presented in Plate 5 are photomicrographs of gastric mucosa from high dose rats obtained on day 7. There was (A) submucosa edema (black arrow) with inflammatory cells infiltration (red arrows). Note vascular congestion (white arrows) H&E X100. (B) shows focal area of surface epithelial mucin production (white arrow) and intracellular mucin production (black arrow) PAS X100.

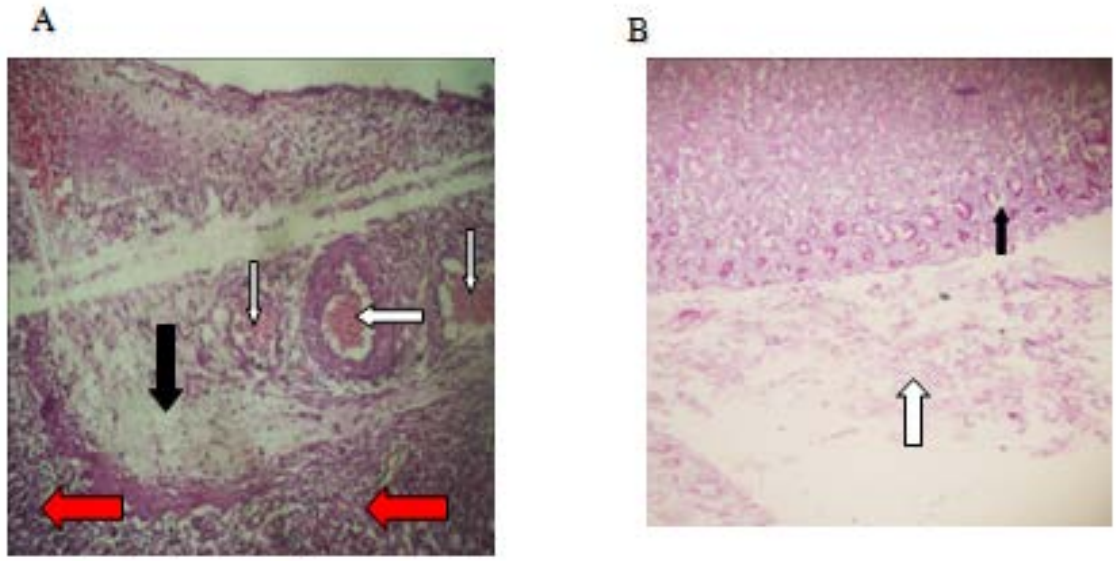


Plate 5: Photomicrographs showing gastric mucosal sections from high dose rats (day 7)

Presented in Plate 6 are photomicrographs of gastric mucosa from high dose rats obtained on day 14. There was (A) granuloma formation lesion H&E X100. (B) section showing mild infiltration of mucosa epithelia (white arrows). The muscular layer (black arrows), submucosa layer (blue arrow) are not infiltrated by inflammatory cells H&E X100.

A **B**

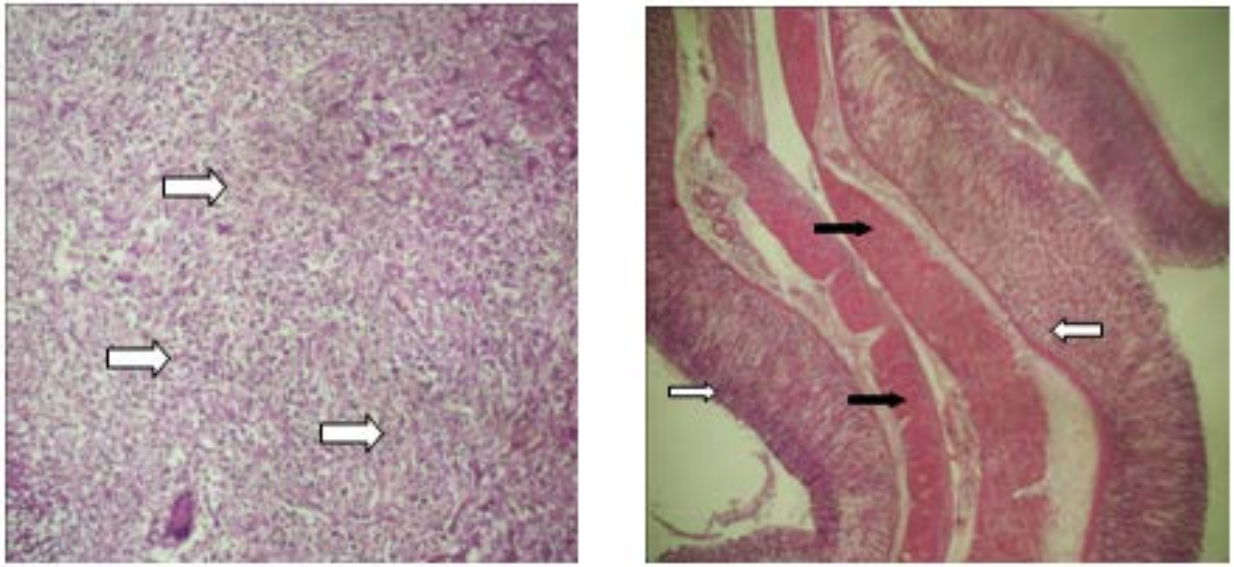


Plate 6: Photomicrographs showing gastric mucosal sections from high dose rats (day14)

Presented in Plate 7 are photomicrographs of gastric mucosa from control rats obtained on day 14. There was (A) focal squamous metaplasia (black arrow) with surrounding lymphoid aggregate (white arrows). H&E X100, and (B) showing focal area of intracellular mucin production, no surface mucin. PAS X100.

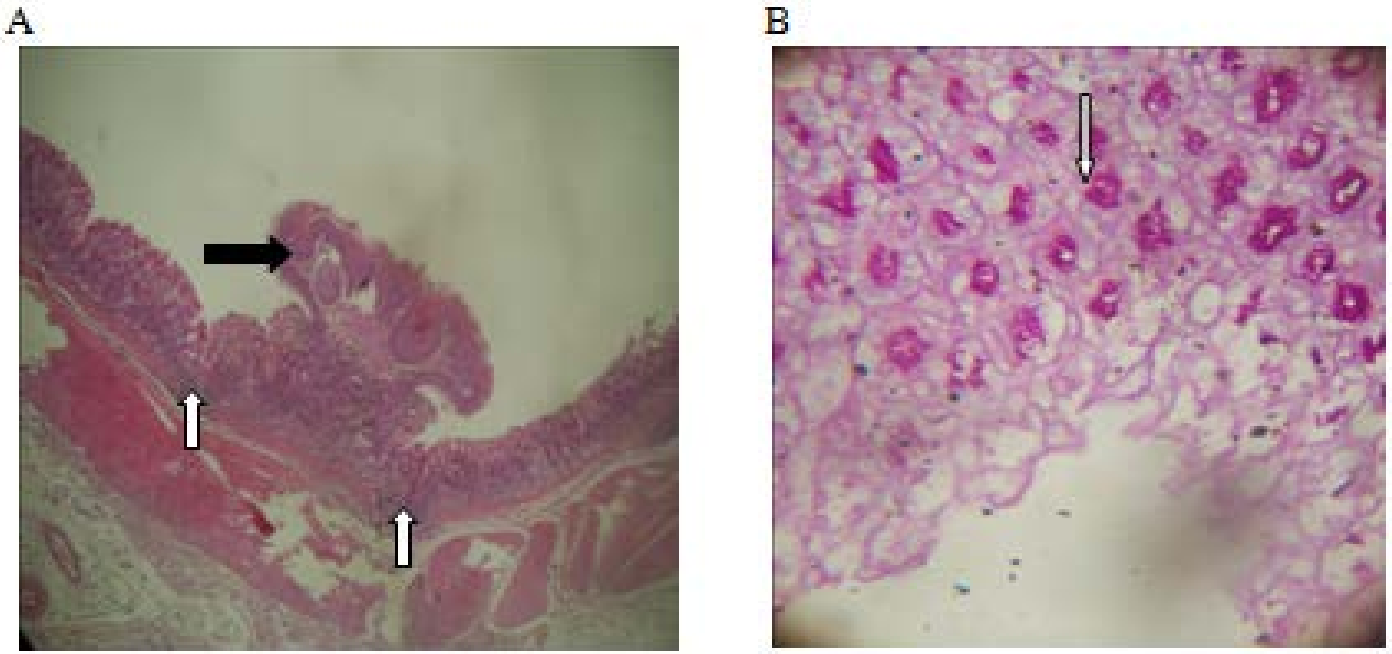


Plate 7: Photomicrographs showing gastric mucosal sections from control rats (day 14)

Presented in Plate 8 are photomicrographs of gastric mucosa from low dose rats obtained on day 14. There was (A) focal granulation tissue formation H&E X100 and (B) section showing focal area of surface epithelial mucin (white arrow) and intracellular mucin production (black arrow). PAS X100.

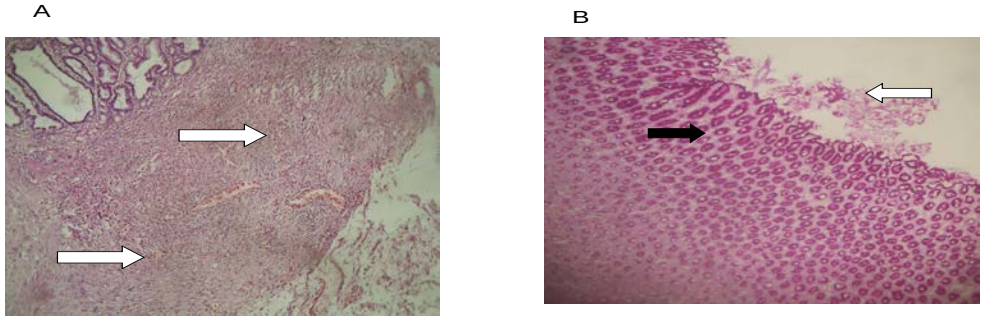


Plate 8: Photomicrographs showing gastric mucosal sections from low dose rats (day 14).

Presented in Plate 9 are photomicrographs of gastric mucosa from control rats obtained on day 21. There was (A) submucosa vascular congestion (white arrows) and infiltration of lamina propria by inflammatory cells (black arrows) healing is almost complete. H&E X100; and (B) showing heavy surface epithelial mucin production (white arrow), mucosa is almost intact. PAS X100.

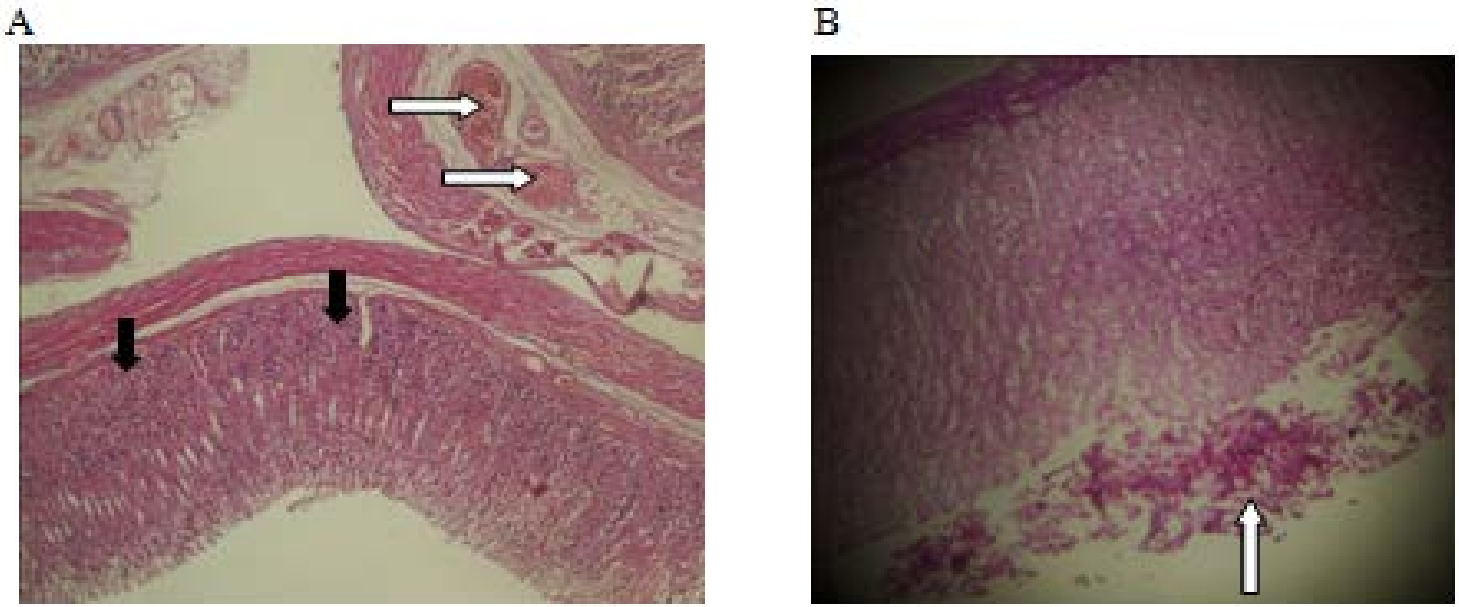


Plate 9: Photomicrographs showing gastric mucosal sections from control rats (day 21).

Presented in Plate 10 are photomicrographs of gastric mucosa from control rats obtained on days 7 and 14. It shows ulcer width and depth in control rats stomach tissue on days 7 (A) and 14 (B) respectively. Note the reduced ulcer depth on day 14 compared to day 7 H&E X100.

A **B**

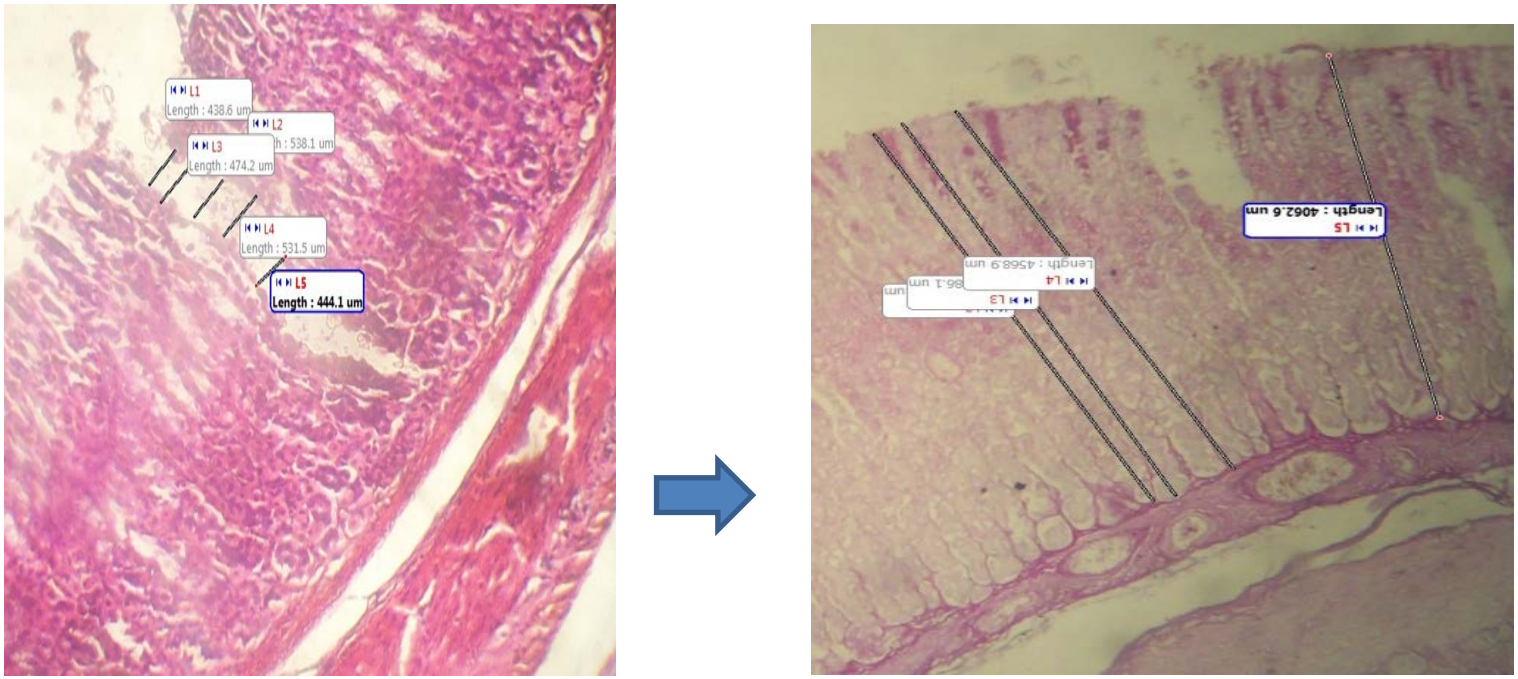
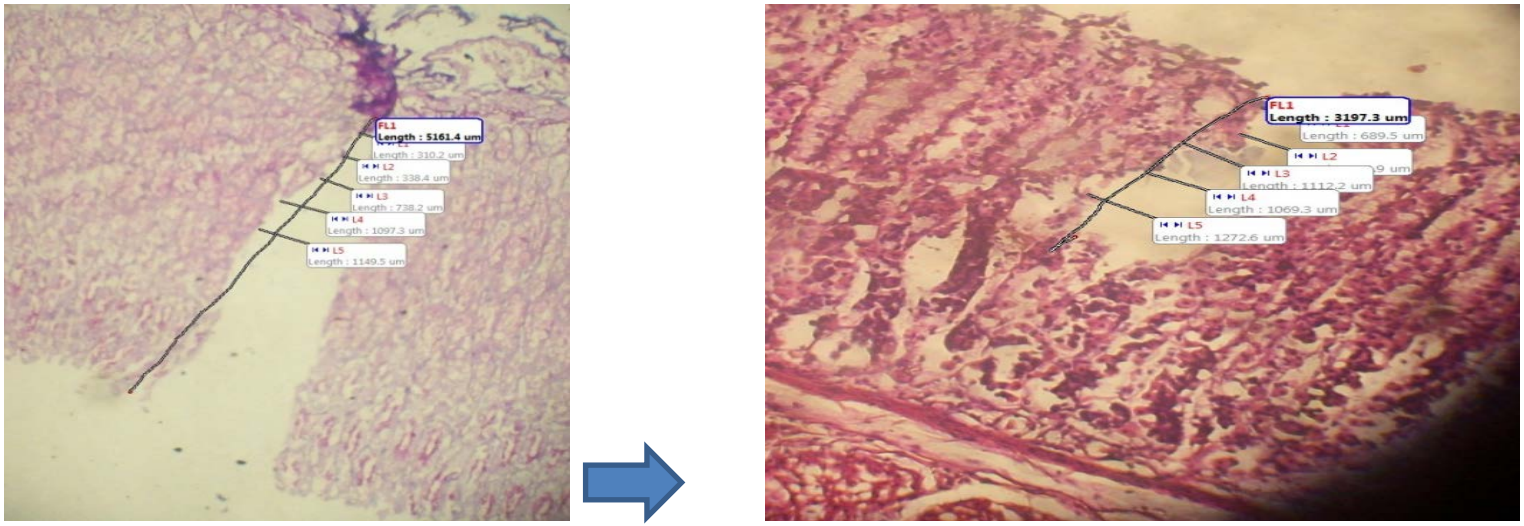


Plate 10: Photomicrographs showing gastric mucosal sections from control rats (days 7 and 14)

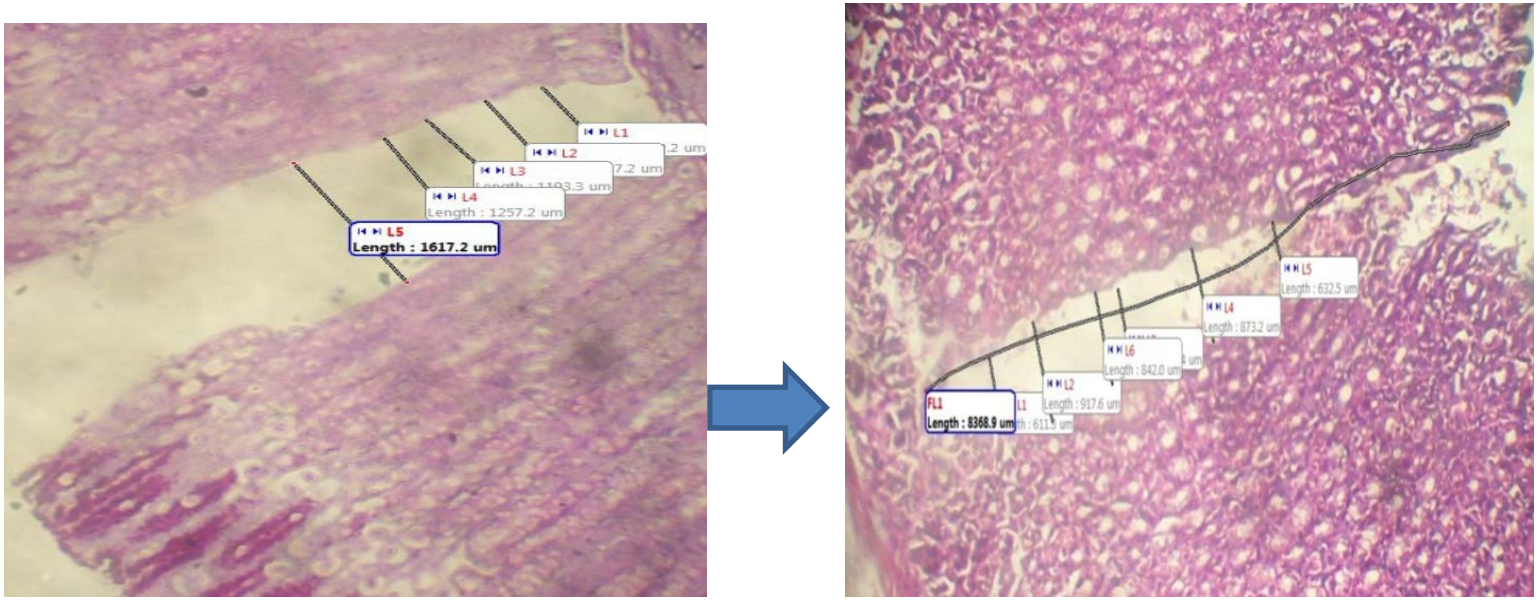
Presented in Plate 11 are photomicrographs of gastric mucosa from low dose rats obtained on days 7 and 14. It shows ulcer width and depth in low dose group rat stomach tissues on days 7 (A) and 14 (B) respectively. Note the deep defect caused by the acetic acid and partial filling of defect by day 14. H&E X100.



A **B**

Plate 11: Photomicrographs showing gastric mucosal sections from low dose group rats (days 7 and 14)

Presented in Plate 12 are photomicrographs of gastric mucosa from high dose rats obtained on days 7 and 14. It shows ulcer width and depth in high dose group rats stomach tissue on days 7 (A) and 14 (B) respectively. Note the deep defect caused by the acetic acid and partial filling of defect by day 14; note also the narrowing of the ulcerated area on day 14. H&E X100.

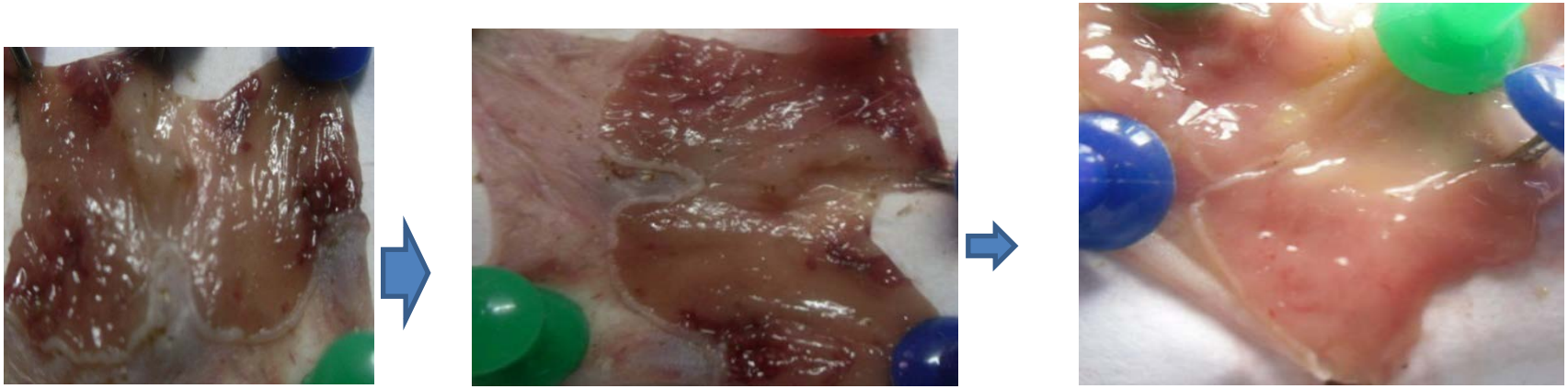


A **B**

Plate 12: Photomicrographs showing gastric mucosal sections from high dose group rats (days 7 and 14)

4.12: Gross morphological appearance of control, low dose and high dose ulcerated rat stomach tissues during ulcer healing

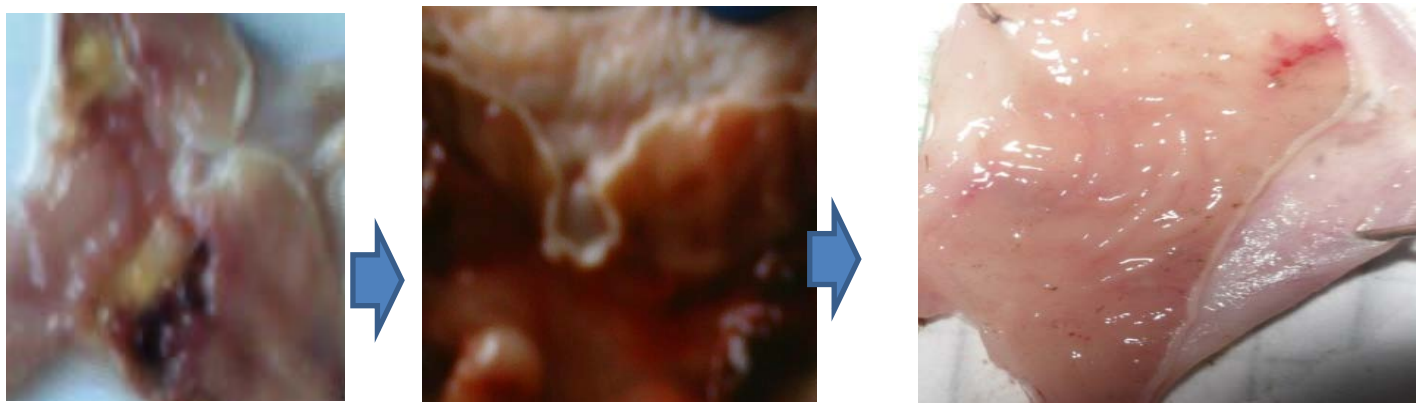
Presented in Plate 13 are photographs of gastric tissue from control rats obtained on days 7, 14 and 21. It shows ulcerated areas on days 7 (A) and 14 (B) and 21 (C) respectively. Note that healing is almost complete on day 21.



A **B** **C**

Plate 13: Photographs showing gastric mucosa from control group rats (days 7, 14 and 21)

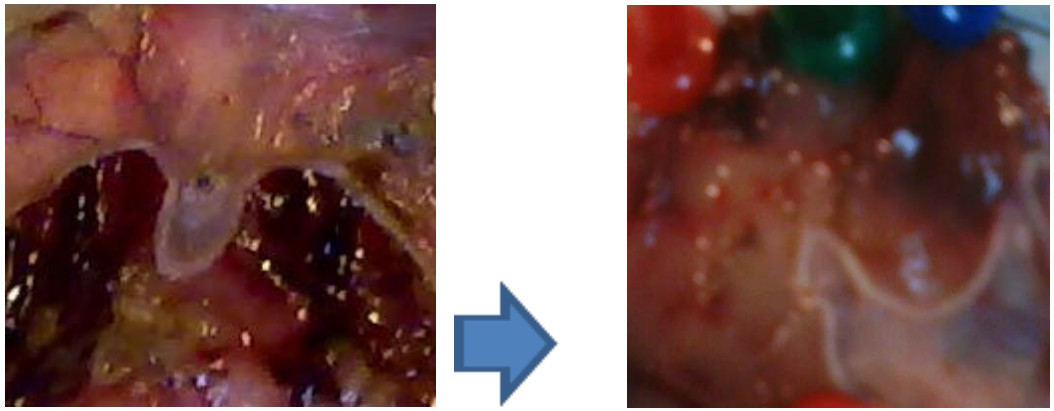
Presented in Plate 14 are photographs of gastric mucosa from low dose rats obtained on days 7, 14 and 21. It shows ulcerated areas on days 7 (A), 14 (B) and 21 (C) respectively. Note that healing is better on day 21 compared to day 14.



A **B** **C**

Plate 14: Photographs showing gastric mucosa from low dose group rats (Days 7, 14 and 21).

Presented in Plate 15 are photographs of gastric mucosa from high dose rats obtained on days 7 and 21. It shows ulcerated areas on days 7 (A) and 21 (B) respectively. Note that healing is delayed compared to control rats.



A B

Plate 15: Photographs showing gastric mucosa from high dose group rats (Days 7 and 21).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

The result from this study showed that animals exposed to high dose of lead in their drinking water for twenty weeks gained less body weight than unexposed controls. In animals exposed to low dose of lead there was no significant difference in body weight gained compared to control. This correlates with the work of Schwartz et al., (1986) and Olaleye *et al.,* (2006). In both works, lead exposure caused decreased body weight gain compared to control.

The significant difference in blood lead levels in both low and high dose groups compared to control group was probably expected and meaningful. The significant difference in plasma lead levels of low dose group compared to high dose group implied that the higher the exposure level to lead, the more the quantity that will be absorbed and stored in the body; and the more the damage that lead will cause to the body. This correlates well with similar work by Olaleye *et al.,* (2006) and Vahedian, (2011) in which they reported that lead exposed animals had higher plasma lead levels than control.

After ulcer induction on day 0, ulcer areas was of equal value in all groups when measured by planimetry. However when measured by histomorphometry a dose-dependent increase in both ulcer width and gastric mucosal area eroded was seen. As healing progressed, (days 7- 21) there was significant difference in the ulcer areas of treated groups compared to control when measured by both planimetry and histomorphometry. This result correlates with similar experiments done by Olaleye *et al.,* (2006) in which they reported that lead exposure causes increase in mean lesion score of ulcers induced by HCl/Ethanol mixture. However this study does not agree with lesion score of ulcers produced by both restraint stress and indomethacin.

This study agrees with the work of Carmouche *et al.*,(2005), in which they reported that lead exposure delays bone fracture healing in mice compared to control (Carmouche *et al.,* 2005).

As healing progressed between days 14 and 21, control group had the highest percentage reduction in ulcer area (39% and 82% respectively compared with ulcer area on day 7) compared to the treated groups. In the treated groups, the percentage reduction were lower than control on both days, however the differences were not significant except for low dose day 14.

In this study, chronic lead exposure caused increase in indomethacin-induced total ulcer score at both low and high doses compare to control. This correlates with the work of Olaleye *et al.,* (2006) in which it was reported that lead exposure for 15 weeks caused increased lesion scores of indomethacin- and HCl/Ethanol-induced ulcerations in rats.

This study showed that chronic lead exposure did not have any significant effect on parietal cell mass in rats at low dose. However at high doses there was significant increase in parietal cell mass compared to control. This increase corresponded with the increased basal gastric acid secretion seen in both acetic acid- and indomethacin-induced ulcerations. This study correlates with the work of Olaleye *et al., (*2011) in which they stated that animals with higher parietal cell mass produce more gastric acid than control, however since the increase in parietal cell mass were minimal, the corresponding increased gastric acid secretion was also only slightly increased.

This study showed that on day 0, there was no significant difference between basal gastric acid secretion of the treated groups and control. As healing progressed (days 7-21) gastric acid secretion decreased progressively in all groups. On days 7 and 14, there were significant

differences between control and high dose group but not between control and low dose groups i.e there was more acid output in treated animals compared to control

On day 21, there were significant differences in basal gastric acid secretion between the control and both treatment groups. In histamine stimulated acid secretion, there was higher acid output in treated animals on day 21 compared to control. In indomethacin-induced ulcerated rats, higher gastric acid output was seen in lead-exposed rats compared to control. The higher gastric acid output in high dose animals seen during healing may be partially responsible for the delayed ulcer healing seen in lead exposed animals. This result correlates with the work of Adeniyi (2012) in which it was stated that as ulcer healing progressed, there is a corresponding decline in both basal and peak histamine-stimulated gastric acid secretion. This result correlates with the work of Vahedian *et al.,* (2011) in which it was stated that lead exposure causes increased nitric oxide (NO) levels in stomach tissue of rats. The increased NO levels correlated with increased acid output in exposed rats, the increased acid output delays ulcer healing by inhibiting cell migration and maturation of the granulation tissue (Schmassmann 2010).

This study showed that chronic lead exposure decreases goblet cell population in the gastric mucosa of rat stomachs compared to controls. During healing in control animals, goblet cell count increased to a maximum value of 55.2 on day 14. In the lead treated groups, there was also increased goblet cell counts but to a lesser extent. The lesser increase in goblet cells count seen in the lead-treated groups may be partly responsible for the delayed ulcer healing seen in the stomachs of the animals in these groups. The result of this study correlated with the work of Olaleye *et al.,* (2006) in which it was stated that gastric barrier mucus of control animals was higher compared to lead-treated animals using three models of ulcer induction viz: restraint stress, HCl/Ethanol mixture and indomethacin induced ulcer models (Olaleye *et al.,* 2006). The

goblet cells secrete mucus that protect the gastric mucosa from the effect of gastric acid and pepsin which are the major causative factors for ulcers. The significantly lower goblet cell counts seen in treated animals may be one of the mechanisms through which lead exposure delays ulcer healing in rats since the mucus produced by these cells are very important in protecting the gastric mucosa from the aggressive factors during healing.

There were no significant changes seen in the plasma concentrations of sodium ion, urea, calcium ions, chloride ion and bicarbonate in the course of the study. However, there was significant difference in potassium ion concentration of high dose group compared to control group. Taken together lead exposure especially at low dose did not alter significantly the plasma biochemical parameters studied in this work. This study agreed with previous reports from several authors such as Truckenbrodt *et al*., (1984) (calcium ion); Herman *et al.,* (2007); (sodium ion and chloride ion) Onuegbu *et al.,* (2007) (bicarbonate and potassium ion) but disagrees with Alasia *et al.,* (2010) (urea).

This study showed that in control animals, red blood cell count increased steadily and significantly during ulcer healing in rats. In the lead-exposed animals, there was no significant increase in red blood cell count during ulcer healing. The slowed rate of increase in red blood cell count during healing in the lead exposed animals is supported by the fact that lead is known to negatively affect erythropoiesis thus predisposing to microcytic hypochromic anemia. Lead closely mimics iron, zinc, calcium, etc. (Bento *et al*, 2002) and within the hemoglobin of red blood cells, it displaces iron causing hypochromic anemia. Lead interferes with the regulation of oxygen transport and energy generation in most tissues of the body (Drill *et al.,* 1979). This may partially explain the delayed healing seen in lead-treated animals since less red blood cells means lesser availability of oxygen to be transported to the ulcer site for healing. The result

from this study agreed with the work of Noori *et al.,* (2003); Sharma and Pandey, (2010) and Alhassan *et al.,* (2010) where they reported that chronic lead exposure caused decreased red blood cell count in male mice and rats respectively.

Results from this study showed that packed cell volume (PCV) increased faster in control than in lead-treated animals during ulcer healing. This is expected because of the increased red blood cell count that was previously observed. The higher PCV level seen in control compared to lead-treated groups correlates well with the red blood cell count. This is expected since red blood cells constitute about 99% of PCV. This result agrees with the work of Noori *et al.,* (2003) and Sharma and Pandey, (2010) where they stated that chronic lead exposure caused decreased PCV in male rats and mice respectively.

This study showed that chronic lead exposure had no clear effect on white blood cell (WBC) count in rats. In the high dose group on day 21, the values were higher than corresponding control. This agreed with the work of Noori *et al* (2003), where it was reported that lead exposure causes increased WBC count in male rats. In the low dose group, on day 0, the value of WBC was lower than the corresponding control. This agreed with the work of Sharma and Pandey, (2010) in which they reported that lead exposure caused a decrease in WBC count in male mice.

This study showed that chronic lead exposure does not have any defined effect on platelet count during healing in gastric ulcerated rats. This work does not agree with the work of Noori *et al,* (2003), where they reported that lead exposure caused increased platelet count in male rats.

This study showed that chronic lead exposure caused increased neutrophil count as a percentage of WBC count in rats. This result is in agreement with the work of Noori *et al* (2003), where they reported that lead exposure caused increased percentage of neutrophil in WBC count in male rats. It also agreed with the report of Dilorezo *et al.*,(2006) in which they showed that lead exposed workers have higher plasma neutrophil count compared to controls in a dose dependent manner.

This study showed that neutrophil/lymphocyte ratio (NLR) was lower in high dose group compared to control throughout. This however, does not agree with the work of Noori *et al* 2003, where they reported that lead exposure causes increased NLR in male rats.

This study showed that gastric MDA activity in the treated groups were higher than in control. This study is in agreement with the work of Harvey *et al, (*1997); Kim *et al* (2006) and Olaleye *et al,* (2007) in which they noted a positive correlation between lead exposure and MDA levels in rats and humans (after 3 months of lead exposure) and Wang *et al,* (2002) in which they reported that after 14 days of lead exposure, treated rats had higher brain MDA content than controls.

This study showed that on day 0, superoxide dismutase (SOD) activity was higher in lead treated animals than control. During healing there was no difference between the treated and control groups. This agreed with the work of Vaziri *et al* (2003) in which they reported increased SOD activity in lead–exposed animals compared to control, but it disagrees with the work of Patil *et al,* (2006) who stated that chronic lead exposure caused decreased SOD activity in erythrocytes of humans. Wang *et al.,* (2002) reported that that lead exposure caused decreased brain SOD activity in rats after 30 days. The differences may be as a result of the different durations of lead

exposure in the animals since in this study, the animals were exposed for 150 days and the different tissues used for the assays.

This study showed that there was no significant difference in catalase activity throughout the duration of the study. This result agrees with the work of Kasperczyk *et al.,* (2004) where it was reported that there were no significant changes in activity of catalase in people chronically exposed to lead and controls. This result does not agree with the work of Olaleye *et al,* (2007) in which it was reported that chronic lead exposure decreases catalase activity in gastric mucosa of rats.

This study showed that chronic lead exposure caused increased apoptosis in the gastric mucosal epithelial cells of exposed animals compared to control. The result from this study correlated with the work of Sharifi., *et al* (2002) and Fox *et al.,* (1998). In both acute studies of lead exposure, lead caused significant levels of apoptosis in the exposed cells compared to control. This result also agreed with that of Ahmed *et al.,* (2012), who showed that 8 weeks of lead exposure caused significant DNA damage and increased apoptosis in the cells of ovaries, lungs, heart, kidneys and liver of female rabbits. Acute gastric mucosal injury is often accompanied with decreased cellular proliferation and increased apoptosis, while cell apoptosis decreases during the healing of gastric ulcers (Zhang *et al*, 2003). This suggests that inhibition of cellular apoptosis is helpful for preventing stress ulcers (Shou-Xiang *et al*., 2007). The results from this study correlated with the above stated works of Zhang *et al.,* (2003).

Conclusion

It can be concluded therefore from this study that twenty weeks of exposure of male albino Wistar rats to lead in form of lead acetate caused delayed healing of experimental (acetic acid-induced) gastric ulceration via delayed/slowed immune response, decreased oxygen supply (via decreased red blood cell count), increased gastric acid secretion, increased lipid peroxidation, increased oxidative stress, decreased mucus production and increased apoptosis in the cells of the stomach of the lead-exposed rats.

Some of the limitations of this study were inability to measure certain parameters that would have shed more light on the various processes/factors involved in ulcer healing such as Angiopoietins, Prostaglandin E_2, interleukins, tissue necrotic factor-alpha, serum response factor, endothelium derived growth factor, etc. Also I was unable to determine angiogenesis during ulcer healing in gastric mucosal tissue. Furthermore, I was unable to measure actual blood flow to ulcerated portions of the gastric mucosa during healing and the arteriovenous oxygen difference of such blood. I was not able to measure epithelial cell proliferation and migration as well during healing. Finally, I was unable to measure the concentrations of pepsin and other gastric proteolytic enzymes present in gastric juice. Since these enzymes are also involved (together with gastric acid) in initiating and sustaining ulcerations, measurement of these would have shed more light on ulcer formation and healing processes. Advanced molecular biology techniques such as flow cytometry, western blotting, etc can be used to determine these parameters and more. These are the areas of possible future research outside the country during post-doctoral training/research.

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APPENDIX

Average weekly Body Weight Changes in rats (g)

Week	Control (g)	low dose (g)	high dose (g)
1	100	101	105
2	101	114	115
3	113	129	133
4	115	124	132
5	126	133	148
6	124	129	162
7	129	127	142
8	130	133	137
9	134	145	147
10	140	161	162
11	144	165	172
12	151	172	180
13	158	176	174
14	164	179	169
15	171	178	168
16	178	176	171
17	182	180	172
18	187	181	177
19	191	184	183
20	203	185	186

Effects of lead on final body weights after 20 weeks of exposure.

Groups	**Control**	**Low dose**	**High dose**
Mean body weight (g)	203.00	184.69*	186.33*
SEM	1.25	5.11	4.18

Levels of plasma lead in different groups of rats after lead exposure for 20 weeks.

Control (microgram/L)	Low dose (microgram/L)	High dose (microgram/L)
0.012	0.488	0.984
0.007	0.511	0.783
0.009	0.541	1.135
0.013	0.654	1.11
0.01	0.586	1.011
0.01	0.604	0.928
Mean 0.0102	**0.564**	**0.992**
S.D 0.0021	0.0621	0.1285
SEM 0.000857	0.0254	0.0525

Packed cell volume in different groups after lead exposure for 20 weeks.

PCV	Control	Low dose	High dose
Day 0	40.4	36.2	29.6
Day7	40.8	36.8	31.4
Day 14	46.6	36.8	33.6
Day 21	48.6	38.8	35.8

SEM for Packed cell volume

SEM PCV	Day 0	Day 7	Day 14	Day 21
Control	2.22	2.13	2.95	1.71
Low dose	1.07	0.86	0.97	0.58
High dose	0.68	0.69	1.77	1.32

White blood cell count (WBC) in different groups after lead exposure for 20 weeks. ($x10^6$)

	Day 0	Day 7	Day 14	Day 21
Control	8510	8530	8860	8050
Low dose	5700	6980	6920	7550
High dose	8780	8240	8620	9840

SEM values for White blood cell count

	Day 0	Day 7	Day 14	Day 21
Control	666.5	160.7	477.2	317.9
Low dose	761.4	413.4	940.2	485.7
High dose	525.4	397.8	960.7	680.8

Superoxide dismutase value in different groups after lead exposure for 20 weeks

SOD	Day 0	Day 7	Day 14	Day 21
Control	1.51	1.83	1.826	1.83
Low dose	1.94	1.82	1.77	1.83
High dose	1.96	1.88	1.92	1.92

SEM for Superoxide dismutase

SOD(SEM)	Control	Low dose	High dose
Day 0	0.022	0.004	0.005
Day7	0.009	0.011	0.005
Day 14	0.0027	0.011	0.003
Day 21	0.006	0.022	0.006

Percentage change in ulcer area in different groups after lead exposure for 20 weeks

	Day 0	Day 7	Day 14	Day 21
Control	100	175.6	107.1	32.44
Low dose	100	221.78	177.33	64.89
High dose	100	319.11	232.44	86.22

Effects of lead exposure on total ulcer scores.

	Control+indo	Low dose+indo	High dose+indo
Mean	12.58	15.91	24.25
SEM	1.3	2.1	1.55

Percentage change in basal acid output in different groups after lead exposure for 20 weeks

	Day 0	Day 7	Day 14	Day 21
Control	100	62.2	52.1	39.5
Low dose	100	73.6	55.2	60
High dose	100	86.4	78.4	69.6

Percentage change in peak acid output in different groups after lead exposure for 20 weeks

	Day 0	Day 7	Day 14	Day 21
Control	100	81.9	68	48.3
Low dose	100	81.6	69.2	71.6
High dose	100	92.7	85.1	75.1

Gastric SOD activity in ulcerated lead-exposed animals

	Day 0	Day 7	Day 14	Day 21
Control	1.51	1.83	1.826	1.83
Low dose	1.94	1.82	1.77	1.83
High dose	1.96	1.88	1.92	1.92

SEM values for SOD activity.

	Control	Low dose	High dose
Day 0	0.022	0.004	0.005
Day7	0.009	0.011	0.005
Day 14	0.0027	0.011	0.003
Day 21	0.006	0.022	0.006

Effects of lead exposure on lipid peroxidation (mean)

	day 0	day 7	day 14	day 21
control	4.93	12.47	10.22	5.39
lowdose	5.8	13.16	11.34	7.89
highdose	*6.65*	*40.54*	*25.59*	*20.45*

SEM

	day 0	day 7	day 14	day 21
control	0.114	0.283	0.335	0.209
lowdose	*0.18*	*0.315*	*0.378*	*0.32*
highdose	0.352	0.613	1.69	0.317

Potassium ion levels in control and lead treated animals (mmol/L).

	Control	Low dose	High dose
Day 0	142.2	143	143.2
Day7	144.6	140.6	140.2
Day 14	141.6	135	137.2
Day 21	140.2	143.2	133.8

Bicarbonate ion levels in control and lead treated animals (mmol/L).

	Control	Low dose	High dose
Day 0	105.6	103.4	138.6
Day7	103.6	99.6	96.8
Day 14	102.2	97.8	98.8
Day 21	100.8	102	95.2

Urea levels in control and lead treated animals (mg/dL).

	Control	Low dose	High dose
Day 0	5.14	5.04	12.54
Day7	4.72	5.28	10.68
Day 14	5.22	5.32	9.2
Day 21	4.9	5.8	11.2

Ca2+ levels in control and lead treated animals (ppm).

	Control	Low dose	High dose
Day 0	19	20.8	21.8
Day7	20.4	21	20.6
Day 14	22.8	22.2	19.6
Day 21	18.2	21.8	20.8