THE EFFECTS OF CHRONIC EXPOSURE TO CHROMIUM SALTS ON GASTRIC ULCERATION IN MALE WISTAR RATS

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CERTIFICATION

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DEDICATION

This project work is dedicated to the ALMIGHTY GOD.

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ABSTRACT

The gastrointestinal tract is constantly exposed to various protective and aggressive factors from food Recent studies have shown that factors, including heavy metal exposure and diet may alter gastrointestinal mucosal integrity. Chromium (Cr), a naturally occurring polyvalent element found in rocks, soil and gases is known to be present in whole grains, wheat, cereal, lettuce, onions, potatoes, green beans, raw tomatoes and many other food items. The role of Cr in gastrointestinal mucosa protection or erosion is not well studied. In this study, the effects of exposure to tri- and hexavalent Cr on gastric ulcer were investigated in rats.

Sixty male Wistar rats (100-120g) were randomly assigned to six groups of 10 animals each. Four groups were treated with Cr: Chromium III–10 ppm (CrIII-10), Chromium III–100 ppm (CrIII-100), Chromium VI–10 ppm (CrVI-10) and Chromium VI–100 ppm (CrVI-100) while the remaining two groups were non-ulcerated control (nCont) and ulcerated control (uCont). Twelve weeks after Cr administration, experimental gastric ulcers were induced via pylorus ligation (PL) technique (n=5 per group). In the remaining five animals, ulcer was induced by oral administration of indomethacin (40 mg/kg). In both ulcer models, animals were sacrificed four hours after ulcer induction. Blood and stomach biopsies were collected and analysed. Ulcer was assessed based on macroscopic appearance of the stomach using standard ulcer score scale. Lipid peroxidation, catalase and Superoxide Dismutase (SOD) activities of the stomach homogenates were assessed by spectrophotometry. Histology of the stomach tissues were assessed to determine the degree of tissue damage using microscopy. Blood Cr level was estimated using the atomic absorption spectrophotometer. Data were subjected to descriptive statistics and analysed using ANOVA at $\alpha_{0.05}$

The blood Cr level was significantly increased in the Cr treated groups (CrIII-10: 0.12±0.01 ppm; CrIII-100: 0.12±0.01 ppm; CrVI-10: 0.13±0.01 ppm and CrVI-100: 0.22±0.03 ppm) compared with nCont (0.08±0.01 ppm). Ulcer scores were nCont (0.0±0.0; 0.0±0.0), uCont (4.0±0.5; 12.6±0.5), CrIII-10 (1.6±0.2; 10.3±0.5), CrIII-100 (2.8±0.1; 10.8±0.2), CrVI-10 (1.8±0.3, 11.4±0.5) and CrVI-100 (2.8±0.3; 12.1±0.5) for PL and indomethacin, respectively. Various degrees of gastric protection were observed in the two ulcer models on exposure to CrIII-10 (59.4%; 18.7%), CrIII-100 (31.3%; 14.5%), CrVI-10 (56.3%; 9.7%) and CrVI-100 (31.3%; 4.0%). There were significant decreases in gastric acidity in Cr- treated groups (CrIII-10:12.0±1.0; CrIII-100: 26.9±2.3 mEq/L/100g; CrVI-10: 16.0±1.5; CrVI-100: 26.8±1.5 mEq/L/100g) compared with uCont (34.0±1.0 mEq/L/100g) for PL model. Lipid peroxidation levels in both PL and indomethacin ulcer models were significantly higher in uCont (11.9±0.1; 6.3±0.1 nmol/mg) than Cr- treated groups (CrIII-10: 8.2±0.1; 4.9±0.0; CrIII-100: 9.0±0.3; 5.3±0.1 nmol/mg; CrVI-10: 8.9±0.3; 5.6±0.0; CrVI-100: 9.7±0.2; 6.0±0.0 nmol/mg). The activities of SOD and catalase were elevated in Cr- treated groups of both ulcer models relative to uCont. Ulcer score results were further corroborated by histological evaluation which revealed mild erosion of surface epithelium in the Cr- treated groups against visible lesions in uCont.

Both tri and hexavalent Chromium offers protection against gastric ulcer induced by pylorus ligation and indomethacin via reduction of gastric oxidative stress.

Keywords: Chromium and gastric ulcer, Pylorus ligation, Indomethacin-induced ulcer, Gastric oxidative stress

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CHAPTER ONE

INTRODUCTION

The gastrointestinal tract is a continuous tube stretching from the mouth to the anus; providing water, electrolytes and nutrients for the body (Guyton and Hall, 2005). The gastrointestinal tract is exposed to different environmental pollutants such as metals and chemicals from different industries, vehicles, as well as everyday basic human activities such as cooking that contaminates food and water (Upreti *et al*., 2004). These pollutants affect the air we breathe, our water and soil (ICEPR, 2013). Most of these chemicals have no known biological function though some of them are capable of disrupting essential physiological processes within the body. Examples of such chemicals include Arsenic, Cadmium, Lead, Mercury and Chromium (Upreti *et al*., 2004).

The gastrointestinal tract is affected with many disorders which include Peptic Ulcer Disease (PUD), Ulcerative colitis (UC), Gastritis, Pyloric stenosis e.t.c. Peptic Ulcer Disease (PUD) is a chronic disease and a worldwide problem affecting 5-10% of the people during their lifetime (Sabiha *et al*., 2011; Sakat *et al*., 2012). It usually occurs in the stomach and the duodenum (Mahajan and Sanghai, 2009). The ulcer formed in the stomach is called gastric ulcer and that of the duodenum is known as duodenal ulcer. It impairs the quality of life and has a high morbidity and mortality rate (Sai *et al*., 2011). Peptic ulcer disease (PUD) occurs as a result of imbalance between aggressive factors such as *Helicobacter pylori* (Yamaguchi and Kakizoe, 2001; Olaleye and Ajeigbe, 2009), Hydrochloric acid, pepsin, refluxed bile, leukotrienes, Reactive Oxygen Species (ROS), ethanol, non-steroidal anti-inflammatory drugs (NSAIDs) (Wallace, 1992; Peskar and Manci, 1998; Rostom *et al*., 2000; Olaleye and Ajeigbe, 2009; Tuñassay and Herszényi, 2010; Silva and de Sousa, 2011) and defensive factors such as acid-pepsin secretion, bicarbonate production, parietal cell activity, mucus

secretion, mucosal barrier, blood flow to mucosa, nitric oxide, cell regeneration and the release of endogenous protective agents such as prostaglandins and epidermal growth factors (EGFs) (Berglindh, 1977; Repetto and Llesuy, 2002; Amr and Maysa, 2010; Silva and de Sousa, 2011; Sakai *et al.*, 2012). Factors such as alcohol abuse, psychological stress (Mawdsley and Rampton, 2006), tension and smoking (Ma *et al.*, 2000; Olaleye and Ajeigbe, 2009), lead exposure (Olaleye *et al.*, 2007), dietary intake of potential ulcerogens (Ibironke *et al.*, 1997; Olaleye and Ajeigbe, 2009) or drugs which stimulate gastric acid and pepsin secretion have also been implicated in the aetiology of peptic ulcer.

Chromium is a transition element and the first element in group 6 of the periodic table and it is regarded as the 24th most abundant element in the earth crust (Emsley, 2001). It occurs naturally and can be found in rocks, plants, animals, soil, and in volcanic dust and gases (Marouani *et al.*, 2012). Intake of hard water supplies Chromium to the body and cooking with stainless steel cook wares also increases the Chromium content in foods (WHO, 2003). Chromium is present in the environment in several forms and the most common being chromium III also known as trivalent chromium and chromium VI also known as hexavalent chromium (Stoecker, 2004; Marouani *et al.*, 2012). Chromium III can be gotten from fresh vegetables and fruits, meat, grains, yeast, sweet potato, corn, whole grains, beef, liver, poultry, turkey, oysters, shellfish e t c (Anderson *et al.*, 1992) all of which helps in keeping the body healthy. Chromium III is important in the metabolism of glucose, protein and lipid in mammals thus enhancing the function of insulin within the body (Mertz, 1969, 1993; 1998).

Chromium VI on the other hand is found in the environment. It is derived almost totally from human activities. In the gastric environment, ingested hexavalent chromium is efficiently reduced to the trivalent form by the gastric juices secreted and ascorbate (Samitz, 1970, De

Flora *et al.*, 1987; U.S. EPA, 1998; ATSDR, 2000). It can also be reduced to chromium III in the lower respiratory tract by the epithelial lining fluid and by pulmonary alveolar macrophages (Dayao and Paine 2001; ATSDR, 2008). Chromium VI is used in the production of stainless steel, textile dyes and wood preservatives, anti - corrosives, tanning of leather, and conversion coatings (Muhammad and Shahida, 2004). The entry routes of chromium into the human body are through inhalation, ingestion and dermal absorption (IPCS, 1998; ATSDR, 2008). Some of the adverse effects of chromium toxicity include irritation in the nose, bleeding of the nose, and difficulty in breathing, coughing, cancer of the lungs and irritant and allergic contact dermatitis (Polak, 1983; Bruynzeel *et al.*, 1988; ATSDR, 2008) depending on the route of exposure.

The exposure of the gastrointestinal tract to metals such as lead acetate has been implicated in the aetiology of peptic ulcer (Olaleye *et al.*, 2007). Exposure to chromium VI has been reported to cause reproductive toxicity in human and laboratory animals (Li *et al.*, 2001; Danadevi *et al.*, 2003; Subramanian *et al.*, 2006; Marouani *et al.*, 2012). However, reports on the mechanism underlying chromium on peptic ulceration are yet to be elucidated.

1.1 AIM OF STUDY

The aim of this study is to investigate the effects of Chromium (III and VI) on experimental ulceration using two different models (Pyloric ligation and Indomethacin).

1.2 OBJECTIVES OF THE STUDY

The objectives of the study include the following:

- To investigate the effect of Chromium III and VI on the blood Chromium level
- To investigate the effect of Chromium III and Chromium VI on ulcer formation induced by indomethacin and pyloric ligation
- To investigate the effect of Chromium III and Chromium VI on gastric acid secretion
- To investigate the effect of Chromium III and Chromium VI on the microscopic stomach architecture
- To investigate the effect of Chromium III and Chromium VI on biochemical reactions of stomach homogenate from various experimental groups

CHAPTER TWO

LITERATURE REVIEW

2.1 STOMACH

The stomach is a hollow, muscular part of the gastrointestinal tract which is involved in the second phase of digestion. It is distensible and can store up to one litre of food (Sherwood, 1997; Scalon and Sanders, 2006). It lies between the oesophagus and duodenum on the upper left of abdominal cavity. The stomach has two sphincters that keep its contents; the oesophageal sphincter found in the cardiac region and pylonic sphincter located at the pyloric region. The stomach is divided into four sections, each of which has different cells and functions. The sections are:

- Cardia: This is the region where the contents of the oesophagus empty into the stomach
- Fundus: This is the upper curvature of the stomach. It is a storage area.
- Body or Corpus: It is the central region of the stomach and a storage area. In an empty stomach, corpus has many longitudinal folds called *rugae gastricae* (Rhoades and Tanner, 2004)
- Pylorus: This is the lower section of the organ which facilitates emptying of the stomach contents into the small intestine. Most digestion takes place in this part of the stomach (Scalon and Sanders, 2006)

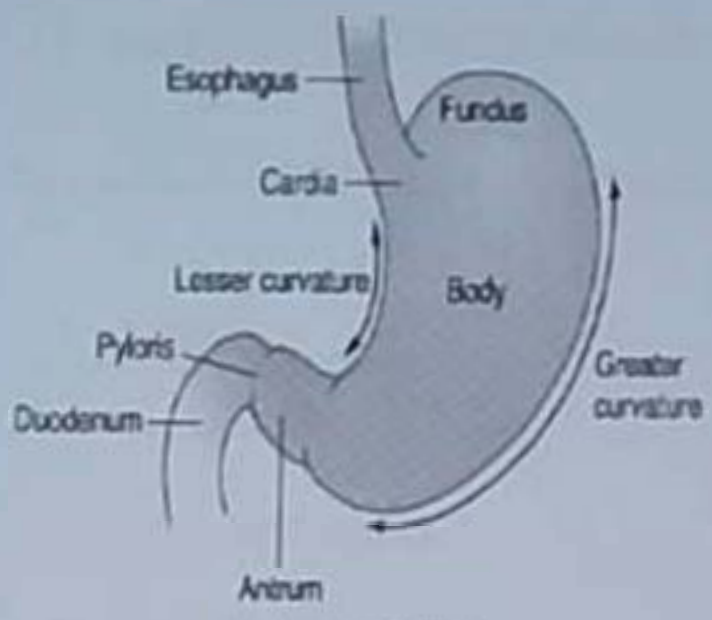


Figure 1: Sections of the stomach

2.1.1 GLANDS OF THE STOMACH

The stomach has three glands which includes

- Cardiac glands located at the Cardia region of the stomach
- Pyloric glands located at the Pylorus part of the stomach
- Fundic glands located at the Fundus of the stomach

The different layers of the stomach glands are lined by different cells such as mucous cells, parietal cells, chief cells and Entroendocrine cells. The mucous cell secretes mucus and bicarbonate ions (HCO_3^-). It helps in protecting the stomach from stomach acid secreted. The parietal cell principally secretes Hydrochloric acid (HCL) and intrinsic factor. It is the most distinctive cell in the stomach. The chief cell secretes pepsinogen and contains zymogen granules

2.1.2 LAYERS OF THE STOMACH WALL

The stomach wall is made up of four layers from inside to outside and these are:

- Mucosa
- Submucosa
- Muscularis externa
- Serosa

Mucosa: It is the first main layer. It consists of the epithelium and the lamina propria. It also has a thin layer of smooth muscle called the muscularis mucosae separating it from the submucosa beneath.

Submucosa: It lies over the mucosa and consists of fibrous connective tissue. It separates the mucosa from the next layer. It is composed of millions of nerve fibres. This layer has a network of nerves called Meissner's plexus or submucosal plexus. This plexus innervate the mucosa and regulate secretions (Senlon and Sanders, 2006).

Muscularis externa: It has three layers of smooth muscles.

i. *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.

ii. *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.

iii. *Outer longitudinal layer:* Auerbach's plexus (or myenteric plexus) is found between this layer and the middle circular layer.

2.1.3 BLOOD SUPPLY TO THE STOMACH

The lesser curvature of the stomach is supplied inferiorly by the right gastric artery and superiorly by the left gastric artery, which also supplies the cardiac region. The greater curvature is supplied inferiorly by the right gastroepiploic artery and superiorly by the left gastroepiploic artery. The fundi of the stomach, and also the upper portion of the greater curvature, are supplied by the short gastric artery.

Figure 2: Schematic image of the blood supply to the stomach (Anne and Moore, 2007).

2.1.4 FUNCTIONS OF THE STOMACH

2.1.4.1 Digestion

Food is churned by the stomach once the bolus enters the stomach by muscular contractions of the wall of the stomach (Richard and Marc, 2007). The boluses are converted into chyme (partially digested food) and this slowly passes through the pyloric sphincter into the duodenum, where the extraction of nutrients begins. The digestion of food into chyme takes place between forty minutes and a few hours depending on the quantity and contents of the meal.

2.1.4.2 Control of Secretion and Motility

The movement and the flow of chemicals into the stomach are controlled by both the autonomic nervous system and by the various digestive system hormones. These hormones include cholecystokinin, secretin, Gastric inhibitory peptide, enteroglucagon and gastrin.

- **Cholecystokinin:** It helps in gall bladder contractions. It decreases gastric emptying and increases release of pancreatic juice which is alkaline and neutralizes the chyme.
- **Secretin:** It diminishes acid secretion in the stomach.
- **Gastric inhibitory peptide:** Gastric inhibitory peptide (GIP) decreases both gastric acid release and gastric motility.
- **Enteroglucagon:** Enteroglucagon decreases both gastric acid secretion and gastric motility.
- **Gastrin:** It causes an increase in the secretion of hydrochloric acid and pepsinogen in the stomach. It also increases motility in the stomach. Gastrin is released by G - cells in the stomach in response to distension of the antrum, and digestive products.

2.1.4.3 Epidermal Growth Factor in gastric defence

Epidermal Growth Factor (EGF) results in cellular proliferation, differentiation, and survival (Herbst, 2004). It is a low-molecular-weight polypeptide found in many human tissues including both submandibular and parotid glands. Salivary EGF plays an important physiological role in the maintenance of oro-oesophageal and gastric tissue integrity. Salivary EGF helps in the healing of oral and gastrooesophageal ulcers, inhibition of gastric acid secretion, stimulation of DNA synthesis and mucosal protection from intraluminal injurious factors such as gastric acid, bile acids, pepsin, and trypsin and to physical, chemical and bacterial agents (Venturi and Venturi, 2009).

2.1.4.4 Stomach as nutrition sensor

The stomach can "taste" sodium glutamate using glutamate receptors (Uematsu *et al*., 2009) and 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), carbobydrates, proteins (Perez *et al*., 1996), and fats (Ackroff *et al*., 2005). This allows the brain to link nutritional value of foods to their tastes (de Araujo *et al*., 2008).

2.1.4.5 Absorption

The absorption of water, simple sugars, amino acids and drugs such as aspirin occurs in the stomach through its lining.

2.2 PEPTIC ULCER DISEASE

Peptic Ulcer is the term used to describe either or both gastric ulcer and duodenal ulcer (Mahajan and Sanghai, 2009; Shirish and Subash, 2012). Peptic ulcer is the most common gastrointestinal tract disorder in clinical practice. It affects approximately 5-10% of the people during their life (Sabiha *et al*., 2011; Sakat *et al*., 2012). Peptic ulcer disease is a worldwide problem. The true prevalence rate of peptic ulcer disease in the Nigerian populace is not certain. Peptic ulcer disease is among the leading causes of morbidity and mortality in Nigeria and many other developing countries (Agbakwuru *et al*., 2006). The occurrence of peptic ulcer is higher among the lower income groups in Nigeria than their counterparts in Europe (Amure, 1967) and higher in the Southern part than in the Northern part (Amure, 1967). It is common in the elderly people of 40years of age and above in Nigeria (Agbakwuru *et al*., 2006).

Gastric ulcer occurs as a result of an imbalance between the aggressive and defensive factors. The aggressive factors include *Helicobacter pylori* (Yamaguchi and Kakizoe, 2001; Olaleye and Ajeigbe, 2009), Hydrochloric acid, pepsin, refluxed bile, leukotrienes, Reactive Oxygen Species (ROS), ethanol, non-steroidal anti-inflammatory drugs (NSAIDs) (Wallace, 1992; Peskar and Marici, 1998; Rostom *et al*., 2000; Lima *et al*., 2006; Olaleye and Ajeigbe, 2009; Tulassay and Herszényi, 2010; Silva and de Sousa, 2011; Sakai *et al*., 2012) and the defensive factors includes acid-pepsin secretion, bicarbonate production, parietal cell activity, mucus secretion, mucosal barrier, blood flow to mucosa, nitric oxide, cell regeneration and the release of endogenous protective agents such as prostaglandins and epidermal growth factors (Berglindh, 1977; Repetto and Llesuy, 2002; Lima *et al*., 2006; Amr and Maysa, 2010; Silva and de Sousa, 2011; Sakai *et al*., 2012). An increase in aggressive factors or a decrease in defensive factors will lead to loss of mucosal integrity resulting in ulceration (Alan *et al*., 1985). Factors such as diet (Lewis and Aderoju, 1978), stress (Pfeffer, 1982), smoking (Doll *et al*., 1958), alcohol consumption (Hagnell and Wretmark, 1957), nutritional deficiency and ingestion of Non-Steroidal Anti Inflammatory Drugs (NSAID's) (Kevin and James, 1985) can all increase the incidence of gastric ulcers. Prolonged anxiety, emotional stress, haemorrhagic surgical shock, burns and trauma have been reported to cause severe gastric irritations (Neelesh *et al*., 2010; Shirisha and Subash, 2012).

The symptoms of peptic ulcer include: Gnawing, burning pain in the upper abdomen (Steven, 2011), heart burn, bloating and abdominal fullness, nausea and copious vomiting, loss of appetite, indigestion and weight loss.

2.2.1 CAUSES OF PEPTIC ULCER DISEASE

2.2.1.1 *Helicobacter pylori*

Helicobacter pylori is a gram negative, spiral shaped, flagellated, bacillus which colonizes the mucus layer of the gastric epithelium (Marshall *et al*., 1985; Tijjani and Umar, 2009). It is the primary cause of ulcers and a common infection worldwide The prevalence rates in the general population ranges from 30-40% in United States, 80-90% in South America and 70-90% in Africa (Martin *et al*., 1989, Kuipers *et al*., 1995; Pounder and Ng, 1995; Oguru *et al*., 1998; Ndububa *et al*., 2001; Tijjani and Umar, 2009). It is more common in developing countries, and its prevalence increases with age from 20% among teenagers to 50-60% of subjects in the 6th and 7th decades of life (Tijjani and Umar, 2009).

H *pylori* was first characterized in 1982 by Robin Warren and Barry Marshall. They described its association with histologic gastritis and subsequently, peptic ulcer disease (Tygat *et al*., 1985; Tijjani and Umar, 2009). In the stomach H. *Pylori* produces on enzyme called urease which neutralizes the stomach acid and thus allow *H. pylori* to thrive in the stomach. *H. pylori* weakens the stomach's defences by thinning the mucous coating of the stomach, making it more susceptible to the damaging effects of acid and pepsin; inflaming the area; poisoning nearby cells and producing more stomach acid (Tijjani and Umar, 2009).

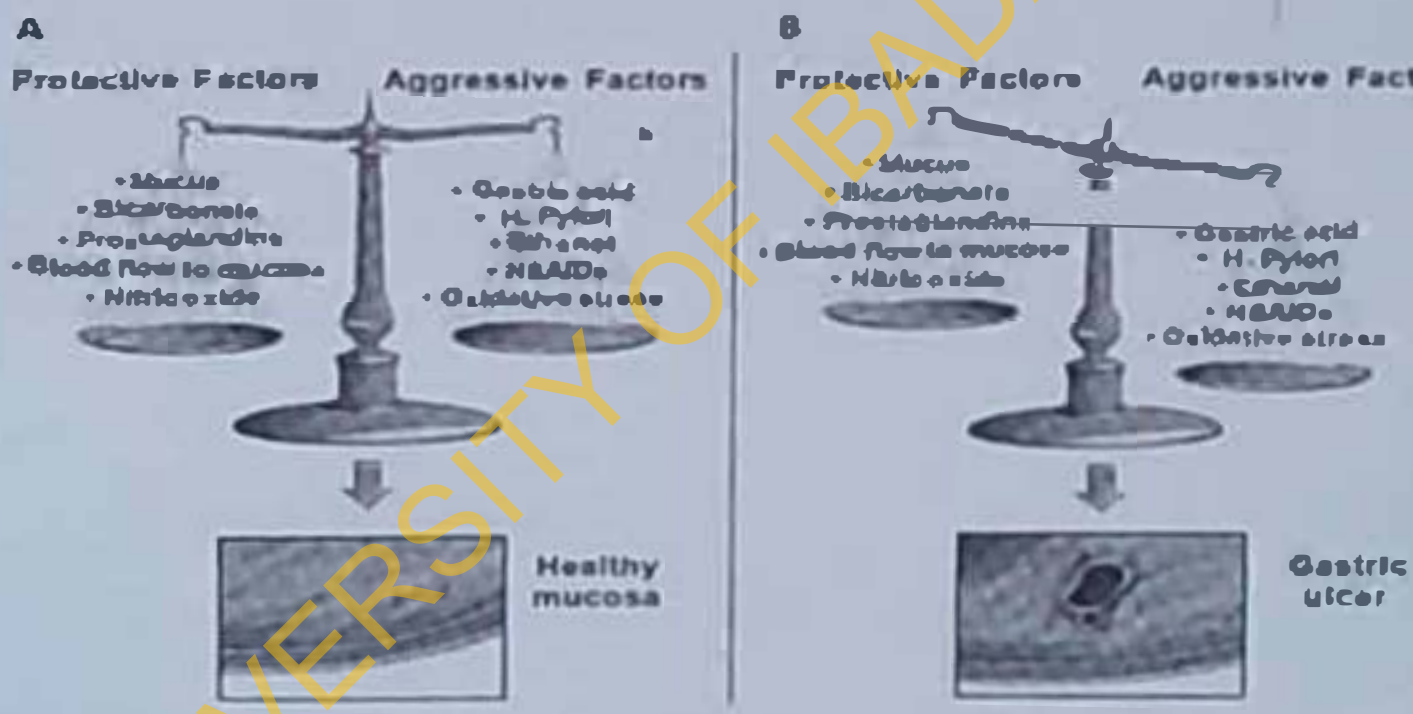
2.2.1.2 Non – Steroidal anti-inflammatory drugs (NSAIDS)

Non- steroidal anti- inflammatory drugs are the most common cause of peptic ulcer disease in patients without *H. pylori* infection (Bytzer and Teglbjaerg, 2001). They are among the most commonly used drugs in the world and they include aspirin, ibuprofen, naproxen, indomethacin, oxoprozin, nabumetone etc. They have analgesic, anti -inflammatory and antipyretic actions (Susana *et al*., 2004).

NSAIDs block prostaglandin production by inhibiting cyclooxygenase (COX). The enzyme has two forms, known as COX-1 and COX-2. COX-1 is involved in producing the prostaglandins that protect the stomach lining, while COX-2 is involved in inflammation (Silva and Sousa, 2011). They interfere with the stomach's ability to produce mucus and bicarbonate, thereby resulting in problems ranging from minor discomfort, such as stomach upset, to life-threatening ulcers and bleeding from the stomach or intestine.

2.2.1.3 Stress

Stress causes the production of excess stomach acid, thus causing ulcer. A study of peptic ulcer patients in a Thai hospital showed that chronic stress was strongly associated with an increased risk of peptic ulcer (Wachirawat *et al.*, 2003).



Source: (Wallace, 1992, Peskar and Marici, 1998, Tulassay and Herszényi, 2010, Silva and de Sousa, 2011)

Figure 3: Gastric mucosa in both healthy and diseased state.

(A) Healthy gastric mucosa: balance between mucosal aggressive and protective factors.

(B) Gastric ulcer formation: imbalance between mucosal aggressive and protective factors.

2.2.1.4 Other factors

Smoking has been found to be a risk factor in ulcer formation (Kato *et al*., 1992; Kurata *et al*., 1997; Salih *et al*., 2007; Martin *et al*., 1989). Other risk factors in the development of ulcers include diet, alcohol consumption; spice consumption and blood type (Sonnenberg *et al*., 1981; Salih *et al*., 2007).

2.3 EXPERIMENTAL ULCER MODELS

Different experimental ulcer models helps in understanding the actiology of ulcer and screening of antiulcer agents. The ulcer models include:

- Non-steroidal anti-inflammatory drug induced ulcers
- Ethanol induced ulcers
- Pylorus ligation induced ulcers
- Water immersion stress induced ulcers
- Histamine induced ulcers
- Reserpine induced ulcers
- Serotonin induced ulcers
- Acetic acid induced ulcers
- Hydrochloric acid induced ulcers

2.3.1 NON-STEROIDAL ANTI-INFLAMMATORY DRUG (NSAID) INDUCED ULCERS

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are a group of drugs that causes reduction of pain and inflammation in joints and muscles. NSAIDs can cause damage to the gastroduodenal mucosa via several mechanisms. These mechanisms include the topical irritant effect of the drugs on the epithelium, impairment of the barrier properties of the

mucosa, reduction of the synthesis of gastric prostaglandin and gastric mucosal blood flow. The presence of acid in the lumen of the stomach also contributes to the pathogenesis of NSAID-induced ulcers and bleeding, by impairing the restitution process, interfering with haemostasis and inactivating several growth factors that are important in mucosal defence and repair (Wallace, 2000).

Indomethacin and aspirin are the commonly used Non-Steroidal Anti-inflammatory Drugs (NSAIDs) for inducing ulcer. They cause ulcer by inhibiting the synthesis of prostaglandins. Prostaglandins are protective agents for gastric mucosa. Prostaglandins protect the gastric mucosa by producing leukotrienes and bicarbonate ions (Shirisha and Subash, 2012). High doses of NSAIDs cause ulceration (Goodman and Gilman, 1996; Wallace, 2000).

Indomethacin at doses of 20mg/kg (Shirisha and Subash, 2012), 25mg/kg (Özbakis and Gürsan, 2005), 40mg/kg (Ajeigbe *et al.*, 2008) have been reported to cause ulceration in rats.

Aspirin inhibits gastric peroxidase and may increase mucosal hydrogen peroxide and hydroxyl ions level to cause oxidative mucosal damage (Goodman and Gilman, 1996; Shirisha and Subash, 2012). Aspirin, at a dose of 200 mg/kg has been reported to cause ulceration four hours after administration (Goel *et al.*, 1985; Hussain *et al.*, 2009; Sivaraman and Muralidharan, 2010; Shirisha and Subash, 2012).

2.3.2 PYLORUS LIGATION INDUCED ULCER

The ligation of the pylorus part of the stomach stops the passage of gastric contents from the stomach and thus creating acidic medium within the stomach for longer time and thereby producing ulcer (Nair *et al.*, 2010; Shirisha and Subash, 2012).

In pylorus ligation induced ulcers, the animal is first anaesthetized, the abdomen is opened and the pylorus part of the stomach is ligated after which the abdomen is sutured back. After

four hours the animal will be sacrificed and the stomach will be cut opened along the greater curvature to score the ulcer formed (Hussain *et al.*, 2009; Shirisha and Subash, 2012).

2.3.3 ETHANOL INDUCED ULCER

Alcohol is a noxious agent and causes gastric mucosal damage by stimulating acid secretion and increasing serum gastrin levels (Turkdogan *et al.*, 1999). The administration of alcohol orally causes increased secretion of gastric juice and a decrease in mucosal resistance due to which protein content of gastric juice is significantly increased by ethanol. This could be leakage because of plasma protein in the gastric juice with weakening of mucosal resistance barrier of gastric mucosa, this leading to peptic ulcer (Thamotharan *et al.*, 2010). Ethanol given orally at doses of 50% v/v (Toker *et al.*, 2013), 99.80% v/v (Shirisha and Subash, 2012) and 100%v/v (Hussain *et al.*, 2009) has been reported to cause ulceration.

2.3.4 WATER IMMERSION STRESS INDUCED ULCER

Stress can arise from prolonged anxiety, tension, and emotion, severe physical discomfort, haemorrhage and surgical shock, burns and trauma, thereby resulting in severe gastric ulceration. Stress induced ulcers can be induced by forcing rats to swim for 3hours in glass cylinder (height 45cm and diameter 35cm) containing water up to 35cm and maintained at 35°C. Animals would have been fasted for 24 hours prior to the experiment. After 3hours of the experiment, animals will be sacrificed and the stomach will be removed and opened along the greater curvature to score the ulcer formed (Malairajan *et al.*, 2008; Shirisha and Subash, 2012).

2.3.5 ACETIC ACID INDUCED ULCER

Acetic acid induced model is used to induce chronic gastric lesions. Acetic acid causes gastric obstruction within the stomach thus leading to ulceration.

A solution of 0.06ml 50% acetic acid instilled into a cylindrical glass tube of 6mm in diameter is placed on the anterior serosa surface of the glandular portion of the stomach 1cm away from the pyloric end under anaesthesia and allowed to remain for 60 seconds. After removal of the acid solution, the abdomen will be sutured back in two layers and animals will be allowed to recover from anaesthesia, then caged and will be fed normally (Aditi *et al*., 2009; Hussain *et al*., 2009; Shirisha and Subash, 2012).

2.4 CHROMIUM

Chromium (Cr) is a transition element with atomic number 24 and it is the first element in group 6. Chromium is a hard metal that takes a high polish and has a high melting point (ATSDR, 2008). It is highly resistant to oxidation, even at high temperatures. It is also odourless, tasteless, and malleable. It is a salt in which an ion contains both Chromium and Oxygen. It is a biologically *inert* metal (Vijayan, 1997). The name of the element is derived from the Greek word "chrōma" (χρῶμα), meaning colour, because many of its compounds are intensely coloured. Chromium is now recognized as one of 15 trace elements critical for proper physiological functioning of lipid and carbohydrate metabolism.

Chromium has magnetic properties: it is the only elemental solid which shows anti-ferromagnetic ordering at room temperature (and below) and above 38 °C, it transforms into a paramagnetic state (Fawcett, 1988).

2.4.1 OCCURRENCE

Chromium occurs naturally and it is found in rocks, volcanic dust and gases, soils as well as plants and animals (Marouani *et al*., 2012). Chromium is the 24th most abundant element in Earth's crust with an average concentration of 100ppm (Emsley, 2001). The concentrations of Chromium in surface water have been increasing since 1999, with 275µg/l being recorded

near salmon spawning areas in the USA (Farag *et al.*, 2006; Marouani *et al.* 2012). The concentrations range in soil is between 1 and 3000 mg/kg, in sea water 5 to 800 µg/litre, and in rivers and lakes 26 µg/litre to 5.2 mg/litre (Kotaś and Stasicka, 2000). Chromium is mined as chromite ($FeCr_2O_4$) ore (National Research Council, 1974). Electronic devices with flat panel displays and cathode-ray TV tubes contain significant amounts of chromium, as well as many other heavy metals, which, when disposed of by land-fill or incineration, can lead to potential human health toxicity and eco toxicity (Lim and Schoenung, 2010; Marouani *et al.*, 2012).

The most common forms of Chromium are Chromium (0), Chromium (III) also known as trivalent Chromium, and Chromium (VI) also known as hexavalent Chromium).

The behaviour and toxicity of Chromium is strongly dependent on the valency, physical chemical properties of the substance, the particle characteristics and the route of exposure/administration (IPCS, 2006a, 2006b).

2.4.2 APPLICATIONS OF CHROMIUM

Chromium is used in three basic industries

- Metallurgical,
- Chemical, and
- Refractory (heat-resistant applications)

These industries are the most important industrial sources of chromium in the atmosphere (EPA 1998; ATSDR 2000).

2.4.2.1 Metallurgy

In the metallurgical industry, Chromium is an important alloying material for steel (ATSDR, 2008. The high- speed tool steels contain between 3 and 5% chromium. Chromium is an important component of stainless steels (the main corrosion-proof metal alloy) and various metal alloys. Metal joint prostheses made of chromium alloys are widely used in clinical orthopaedics.

Nickel-based alloys increase in strength due to the formation of discrete, stable metal carbide particles at the grain boundaries. For example, Inconel 718 contains 18.6% chromium. These nickel superalloys, they are used in jet engines and gas turbines in lieu of common structural materials because of their excellent high-temperature properties (Bhadeshia, 2009).

The strong oxidative properties of chromates are used to deposit a protective oxide layer on metals like aluminium, zinc and cadmium in the chromate conversion coating process (Edwards, 1997).

2.4.2.2 Chemical Industry

In the chemical industry, chromium is used primarily in

- Dye and pigment

The mineral crocoite (Lead Chromate, $PbCrO_4$) was used as a yellow pigment shortly after its discovery. The pigment does not photo degrade, but it tends to darken due to the formation of Chromium (III) oxide. It has a strong colour, and was used for school buses in the US and for Postal Service (for example Deutsche Post) in Europe. The use of chrome yellow declined due to environmental and safety concerns and was replaced by organic pigments or alternatives free from lead and chromium. Other pigments based on chromium are, for

example, the bright red pigment chrome red, which is a basic lead chromate ($PbCrO_4 \cdot Pb(OH)_2$).

A very important chromate pigment, which was used widely in metal primer formulations, was zinc chromate, now replaced by zinc phosphate. A wash primer was formulated to replace the dangerous practice of pre - treating aluminium aircraft bodies with a phosphoric acid solution. This used zinc tetroxychromate dispersed in a solution of polyvinyl butyral. An 8% solution of phosphoric acid in solvent was added just before application. It was found that an easily oxidized alcohol was an essential ingredient. A thin layer of about 10–15 μm was applied, which turned from yellow to dark green when it was cured. There is still a question as to the correct mechanism. Chrome green is a mixture of Prussian blue and chrome yellow, while the chrome oxide green is chromium (III) oxide (Gettens, 1966).

Chromium oxides are also used as a green colour in glassmaking and as a glaze in ceramics (Gent *et al*., 2005). Green chromium oxide is extremely light-fast and as such is used in cladding coatings. It is also the main ingredient in IR reflecting paints, used by the armed forces, to paint vehicles, to give them the same IR reflectance as green leaves (Marrion, 2004).

- **Wood preservative**

Chromium VI salts are used because of their toxicity, for the preservation of wood. For example, Chromated Copper Arsenate (CCA) is used in timber treatment to protect wood from decay fungi, wood attacking insects, including termites, and marine borers (Hingston *et al*., 2001). The formulations contain chromium based on the oxide CrO_3 between 35.3% and 65.5%. In the United States, 65,300 metric tons of CCA solutions have been used in 1996 (Hingston *et al*., 2001).

- **Leather Tanning**

Chromium (III) salts, especially Chrome alum and Chromium (III) sulfate, are used in the tanning of leather. The Chromium (III) stabilizes the leather by cross linking the collagen fibers (Brown, 1997). Chromium tanned leather can contain between 4 and 5% of chromium, which is tightly bound to the proteins (National Research Council (U.S.), 1974).

- **Catalysts**

Several chromium compounds are used as catalysts for processing hydrocarbons. For example the Phillips catalysts for the production of polyethylene are mixtures of chromium and silicon dioxide or mixtures of chromium and titanium and aluminium oxide (Weckhuysen *et al.*, 1999). Fe-Cr mixed oxides are employed as high-temperature catalysts for the water gas shift reaction (Twigg, 1989; Rhodes *et al.*, 1995). Copper Chromite is a useful hydrogenation catalyst.

- **Chrome Plating**

It is a technique of electroplating a thin layer of chromium onto a metal or plastic object. The chromed layer can be decorative, provide corrosion resistance, ease cleaning procedures, or increase surface hardness.

- **Synthetic ruby and the first laser**

Natural rubies are corundum (Aluminium oxide) crystals that are coloured red (the rarest type) due to chromium (III) ions (other colours of corundum gems are termed sapphires). A red-coloured artificial ruby may also be achieved by doping chromium (III) into artificial corundum crystals, thus making chromium a requirement for making synthetic rubies (Moss and Newnham, 1964). Such a synthetic ruby crystal was the basis for the first laser, produced

in 1960, which relied on stimulated emission of light from the chromium atoms in such a crystal.

2.4.2.3 Refractory material

Chromite and chromium (III) oxide are characterized by high heat resistivity and high melting point and thus they are good material for high temperature refractory applications such as cement kilns, molds for the firing of bricks, blast furnaces and as foundry sands for the casting of metals

2.4.2.4 Other use

- Chromium (IV) oxide (CrO_2) is a magnetic compound. It is used to manufacture magnetic tape used in high-performance audio tape and standard audio cassettes (Mallinson, 1993). Chromates can prevent corrosion of steel under wet conditions, and therefore chromates are added to drilling muds (Garverick, 1994).
- Chromium (III) oxide is a metal polish known as green rouge.
- Chromic acid is used for cleaning laboratory glassware of any trace of organic compounds. Sodium dichromate is sometimes used because of its higher solubility (50 g/L versus 200 g/L respectively). Potassium dichromate is a chemical reagent, used in cleaning laboratory glassware and as a titrating agent. It is also used as a moidant (i.e. a fixing agent) for dyes in fabric.

2.4.3 VALENCE STATES OF CHROMIUM

Chromium exists in a series of oxidation states from -2 to +6 valence. The most important stable states are 0 (elemental metal), +3 (trivalent), and +6 (hexavalent).

The health effects of chromium are primarily related to the valence state of the metal at the time of exposure. Trivalent (Cr III) and hexavalent (Cr VI) compounds are thought to be the most biologically significant.

2.4.3.1 CHROMIUM III

Chromium III is an essential nutrient required by the human body to promote the action of insulin in body tissues. It plays an important role in the maintenance of normal carbohydrate, lipid and protein metabolism (EVM, 2003). It occurs naturally in the environment. About 0.5-1% of Chromium III present in the normal diet is absorbed (IPCS, 2006a; DEFRA and EA, 2002). Trivalent chromium is poorly transported across membranes. Daily exposure from food sources, excluding supplements, is estimated at about 0.1 mg (EVM, 2003). Absorption from the intestines is low (0.5-2%) and is thought to involve a mechanism other than passive diffusion (EVM, 2003).

Chromium III is essential for animals and human beings. Chromium influences glucose, protein, and lipid metabolism. Chromium deficiency may cause changes in the metabolism of glucose and lipids. Chromium deficiency is associated with cardiovascular disease, impaired glucose tolerance and glucose utilisation, fasting hypoglycaemia, impaired fertility, decreased sperm count, weight loss, neuropathy, altered plasma fatty acid profile and nitrogen metabolism, and depressed respiratory quotient (EVM, 2003).

Chromium III is found in most fresh foods and drinking water. Dietary sources rich in Chromium III include breads, cereals, fish, fresh vegetables, meats, and spices. Other

significant sources of Chromium III are mineral supplements, brewer's yeast and beer (ATSR, 2008). The National Research Council has identified an estimated safe and adequate daily dietary intake (ESADDI) for Chromium of 50-200 µg/d (NRC, 1989), corresponding to 0.71-2.9 µg/kg/day for a 70-kg adult. FDA has selected a Reference Daily Intake for Chromium of 120µg/d (DHHS, 1995).

The biologically active form of an organic Chromium III complex, often referred to as glucose tolerance factor (GTF), is believed to function by facilitating the interaction of insulin with its cellular receptor sites. Studies have shown that the Chromium III supplementation in deficient and marginally deficient subjects can result in the rapid reversal of many of the symptoms of chromium-deficiency (Cohen, *et al* 1993; Mertz, 1993; ATSR, 2008).

2.4.3.2 CHROMIUM VI

Chromium VI is also known as hexavalent chromium. It is 100 - 1000 times more toxic than the most common trivalent compounds (EPA, 1998; ATSDR, 2000; Dayan and Paine, 2001 Stoecker, 2004; Marouani *et al.*, 2012). It is a strong oxidizing agent and widely known to cause allergic dermatitis as well as toxic and carcinogenic effects in humans and animals (Von and Liu, 1993; Marouani *et al.*, 2012). Hexavalent Chromium VI in the environment is almost totally derived from human activities (WHO, 1990).

Chromium VI has two main forms (CrO_4^- and $Cr_2O_7^-$). These two forms are dominant in the environment and can readily cross cellular membranes with the help of nonspecific anion carriers (Marouani *et al.*, 2012). Hexavalent Chromium is ultimately reduced to trivalent Chromium inside the cell through the formation of reactive intermediates like pentavalent and tetravalent forms (De Flora *et al*, 1990. Marouani *et al*, 2012).

The LD50 for chromium (VI) ranges between 50 and 150 mg/kg (Katz and Salem, 1992). The acute toxicity of Chromium (VI) is due to its strong oxidational properties. Chromium VI induced acute and chronic toxicity, neurotoxicity, dermatotoxicity, genntoxicity, carcinogenicity, immunotoxicity and general environmental toxicity (Von and Liu, 1993; Li *et al.*, 2011; Marouani *et al.*, 2012).

Soluble and insoluble Chromium VI salts have been demonstrated to induce morphological and neoplastic transformation and mutagenicity in human and murine cells (Paustro *et al.*, 1988; Marouani *et al.*, 2012)

The carcinogenicity of chromate dust is known for a long time, and in 1890 the first publication described the elevated cancer risk of workers in a chromate dye company (Newman, 1890; Langard, 1990). Three mechanisms have been proposed to describe the genotoxicity of Chromium VI. The first mechanism includes highly reactive hydroxyl radicals and other reactive radicals which are by products of the reduction of Chromium VI to Chromium III. The second process includes the direct binding of Chromium (V), produced by reduction in the cell, and Chromium (IV) compounds to the DNA. The last mechanism attributed the genotoxicity to the binding to the DNA of the end product of the Chromium (III) reduction (Cohen, 1993).

Chromium salts (chromates) are also the cause of allergic reactions in some people. Chromates are often used to manufacture, amongst other things, leather products, paints, cement, mortar and anti-corrosives. Contact with products containing chromates can lead to allergic contact dermatitis and irritant dermatitis, resulting in ulceration of the skin, sometimes referred to as "chrome ulcers". This condition is often found in workers that have been exposed to strong chromate solutions in electroplating, tanning and chrome-producing manufacturers (Basketter *et al.*, 2000)

Exposure to Chromium VI has been reported to cause reproductive toxicity in human and laboratory animals (Li *et al.*, 2001; Danadevi *et al.*, 2003; Subramanium *et al.*, 2006; Marouani *et al.*, 2012). A decreased sperm cell count and motility as well as increased follicle stimulating hormone (FSH) serum concentration were found in men employed in electroplating (Multinger *et al.*, 1996; Marouani *et al.*, 2012). Moreover, a decreased concentration of sperm cells and increase in abnormal spermatozoa were observed in mice (Acharya *et al.*, 2006; Marouani *et al.*, 2012), rats (Li *et al.*, 2001; Marouani *et al.*, 2012), rabbits (Yousef *et al.*, 2006; Marouani *et al.*, 2012) and bonnet monkeys (Subramanian *et al.*, 2006; Marouani *et al.*, 2012) treated/exposed to Chromium.

2.4.4 CHROMIUM EXPOSURE AND ABSORPTION

Chromium is one of the most widely used industrial metals and it is one of the major contaminants in various hazardous waste sites worldwide (EPA, 2002; Medeiros *et al.*, 2003). Humans can be exposed to chromium via inhalation, ingestion and dermal absorption (IPCS, 1998; ATSDR, 2008). Absorption of Chromium is dependent on the valence, solubility of the particular Chromium species, gastrointestinal transit time and the chemical form of chromium compounds (ATSR, 2008).

2.4.4.1 Inhalation

The primary target for inhaled Chromium is the respiratory tract (ECB, 2005). Chromium particles inhaled are deposited in the lungs and can be coughed up and swallowed. Chromium deposited deep in the lungs can dissolve and pass through the lining of the lungs and enter the bloodstream. The absorption of chromium after inhalation can be influenced by the size, oxidation state, and solubility of the chromium particles; the activity of alveolar macrophages, and the interaction of chromium with biomolecules after deposition in the lung (ATSDR, 1993).

The amount of chromium absorbed after inhalation can be detected in the serum, urine and hair of exposed individuals (Tossavainen *et al.*, 1980; Randall and Gibson, 1987; Minota and Cavalieri, 1988). Inhaled Chromium is excreted both in the urine and the faeces. After human exposure to Chromium (III) by inhalation, urinary concentrations of Chromium were found to be increased indicating respiratory absorption (Aitio *et al.*, 1984; Foa *et al.*, 1988; Dayan and Paine, 2001). Chromium (VI) compounds are absorbed more readily than Chromium (III) compounds, probably because Chromium (VI) readily penetrates cell membranes (Mertz 1969, Wiegand *et al* 1984). Chromium (VI) is reduced to Chromium (III) in the lower respiratory tract by the epithelial lining fluid and by pulmonary alveolar macrophages (Dayan and Paine, 2001). Chromium VI can be reduced into Chromium III in the epithelial lining fluid of the lungs by ascorbate and glutathione (Petrilli *et al.*, 1986; Suzuki and Fukuda, 1990).

2.4.4.2 Ingestion

The major source of chromium exposure for most people especially in the United States is through food intake. Chromium content of food can be altered by various methods of processing, storage and preparation. Foods prepared with stainless steel cooking utensils might contain higher level of chromium because of leaching from stainless steel. Humans can also be exposed to Chromium through the usage of consumer products such as household utensils, wood preservatives, cement, cleaning products, textiles, and tanned leather (ATSDR, 2000; ATSDR, 2008).

In general, Chromium (VI) compounds are better absorbed through the intestinal mucosa than the Chromium (III) compounds. Ingested chromium VI is converted to chromium III due to the actions of stomach acid and other components within the gastrointestinal tract (Cohen *et al.*, 1993; De Flora *et al.*, 1987). In humans and animals, less than 1% of inorganic

Chromium (III) and about 10% of morgatuc Chromium (VI) are absorbed from the gut; the latter amount is slightly higher in a fasting state (Donaldson and Barreras, 1966; Dayan and Paine, 2001). Once it is absorbed, Chromium is distributed to all parts of the body and then passes through the kidneys and is eliminated in the urine in a few days. The trivalent form in food can attach to other compounds that make it easier for Chromium to be absorbed and enter the bloodstream from the stomach and intestines.

2.4.4.3 Dermal Contact

Data from volunteers and indirect evidence from occupational studies indicate that absorption of Chromium (VI) compounds can occur through intact skin (Baranowska-Dutkiewicz, 1981). Studies in experimental animals showed poor absorption of Chromium (III) compounds following dermal route (Dayan and Paine, 2001).

2.4.5 EFFECTS OF CHROMIUM EXPOSURE ON HUMAN HEALTH AND ANIMAL HEALTH

2.4.5.1 Mechanism of Chromium toxicity

The solubility and the oxidation state of chromium compounds are the major factors governing their toxicity and this toxicity is attributable to chromium VI form, this is because chromium III is poorly absorbed via any route of exposure (ATSDR, 2008).

Chromium VI compounds are powerful oxidizing agents, corrosive and more toxic than chromium III compounds, this can be traced to the ease by which chromium VI can readily pass through the cell membranes.

When chromium VI reduces to chromium III at a distance from the target site for toxic or genotoxic effect, it offers a detoxification process and when the reduction takes place within

or near the nucleus of target organs, it may serve to activate chromium toxicity (Dayan and Paine, 2001; ATSDR, 2008).

The reduction of Chromium VI is considered to serve as a detoxification process when it occurs at a distance from the target site for toxic or genotoxic effect while reduction of Chromium VI may serve to activate chromium toxicity if it takes place in or near the cell nucleus of target organs (Dayan and Paine, 2001). If Chromium VI is reduced to Chromium III extracellularly, this form of the metal is not readily transported into cells and so toxicity is not observed. Chromium VI can be reduced under physiological conditions by hydrogen peroxide (H_2O_2), glutathione (GSH) reductase, ascorbic acid, and GSH to produce reactive intermediates, including Chromium (V), Chromium (IV), thiyl radicals, hydroxyl radicals, and ultimately, Chromium (III) (ATSDR, 2008) and any of these species could attack DNA, proteins, and membrane lipids, thereby disrupting cellular integrity and functions (De Mattia *et al*., 2004; ATSDR, 2008).

The various effects of Chromium on human health and animals are as follows:

2.1.5.2 Respiratory effects

Chromium compounds causes irritation of the airway, obstruction of the airway, lung, nasal, or sinus cancer when inhaled. The adverse health effect seen is dependent on the dose, duration of exposure and the specific compound involved (ATSDR, 2008).

Pulmonary irritant effects following inhalation of chromium dust can include asthma, chronic bronchitis, chronic irritation, chronic pharyngitis, chronic rhinitis, congestion and hyperemia, polyps of the upper respiratory tract, tracheobronchitis, and ulceration of the nasal mucosa (Lindberg and Hedenstierna, 1983; Dayan and Paine, 2001; ATSDR, 2008).

Occupational exposure to Chromium III has also been associated with respiratory effects. One man developed coughing, wheezing, and decreased forced volume after an inhalation exposure to a sample of Chromium (III) sulfate (Novey *et al.*, 1983; ATSDR, 2008). The respimtory system in ammals is also a primary target for inhalation exposure to chromium.

2.4.5.3 Skin Effects

Dermal exposure to Chromium produces irritant and allergic contact dermatitis (Polak, 1983; Bruynzeel *et al.*, 1988; ATSDR, 2008). Primary irritant dermatitis is related to the direct cytotoxic properties of Chromium, while allergic contact dermatitis is an inflammatory response medinted by the immune system. Allergic contact dermatitis is a cell mediated immune response that occurs in a two-step process (induction and sensitization). In the induction stage chromium is absorbed into the skin, this will now trigger the second stage. Sensitized individuals will exhibit an allergic dermatitis response when exposed to Chromium above a threshold level (Polak, 1983; Lewis, 2004; ATSDR, 2008). Chromium allergic dermatitis is characterized by symptoms of dryness, erythema, fissuring, papules, scaling, small vesicles, and swelling (MacKie, 1981; Adams, 1990; ATSDR, 2008).

The primary determinants of the capacity of individual chromium compounds to elicit an allergic response are solubility and pH (Polak *et al.* 1973; Fregert and Fregert, 1981; ATSDR, 2008). The low solubility Chromium (III) compounds are much less efficient contact allergens than Chromium VI (Spruit and van Neer, 1966; ATSDR, 2008).

When chromium penetrates the skin, it can result into painless erosive ulceration called chrome holes with delayed healing (ATSDR, 2008). These commonly occur on the fingers, knuckles, and forearms. The characteristic chrome sore begins as a papule, forming an ulcer with raised hard edges. Ulcers can penetrate deep into soft tissue or becomes the site of

secondary infection, but are not known to lead to malignancy (Deng *et al.*, 1990; Geller, 2001; Lewis, 2004; ATSDR, 2008).

Chromium is one of the most common skin sensitizers and often causes skin sensitizing effect in the general public. A possible source of chromium exposure is waste dumps for chromate-producing plants causing local air or water pollution.

2.4.5.4 Gastrointestinal Effects

Mancuso carried out a study on 97 workers from a chrome plant exposed to a mixture of insoluble chromite ore containing Chromium III and soluble Chromium VI as sodium chromate and dichromate. Gastrointestinal radiography revealed that 10 of the workers had ulcer formation, and of these, six had hypertrophic gastritis. Nearly all of the workers breathed through the mouth while at work and swallowed the chromate dust, thereby directly exposing the gastrointestinal mucosa (Mancuso, 1951; ATSDR, 2008). Most of the previous studies reporting gastrointestinal effects, however, did not compare the workers with appropriate controls.

2.4.5.5 Renal Effects

Renal effects after inhalation or oral exposure to Chromium VI compounds have been reported. Glomerular injury has been noted in chromium workers and the predominant renal injury is tubular. A feature of chromate nephropathy is injury to the brush border membrane (Kirschbaum *et al.*, 1981; ATSDR, 2008).

Severe chromium poisoning can cause acute tubular necrosis and acute renal failure (Sharma *et al.*, 1978; ATSDR, 2008). In chrome platers, elevated urinary β2-microglobulin levels have been found (Lindberg and Hedenstierna, 1983; ATSDR, 2008).

Occupational exposure to Chromium III does not appear to be associated with renal effects (ATSDR, 2000; ATSDR, 2008). No renal impairment based on urinary albumin, retinol binding protein, and renal tubular antigens was found in 236 workers employed in the ferrochromium production industry (Fon *et al*., 1988; ATSDR, 2008).

2.4.5.6 Hepatic Effects

Chromium VI has been reported to cause severe liver effects in four of five workers exposed to chromium trioxide in the chrome plating industry. The reported liver effects include derangement of the liver cells, necrosis, lymphocytic and histocytic infiltration, and increases in Kupffer cells (Pascale *et al*., 1952; ATSDR, 2008).

Cases of hepatic effects after oral exposure to Chromium VI compounds have also been reported. Elevated liver enzyme levels were reported following ingestion of 150 mL solution containing 22.5 g potassium dichrome (Kolacinski *et al*., 1999; ATSDR, 2008). Hepatomegaly (Michie *et al*., 1991; Meert *et al*., 1994; ATSDR, 2008) and hepatic failure (Loubieres *et al*., 1999; Stift *et al*., 2000; ATSDR, 2008) have also been noted in the cases of acute poisoning.

Exposure to Chromium III has not been found to cause any liver effects in workers employed in two factories that produced Chromium III oxide or Chromium III sulfate (Korallus *et al*., 1974b; ATSDR, 2008).

2.4.5.7 Cardiovascular Effects

Study shows that a 22-month-old boy who ingested an unknown amount of sodium dichromate died of cardiopulmonary arrest. Autopsy revealed early hypoxic changes in the myocardium (Ellis *et al*., 1982; ATSDR, 2008). A 15-year-old woman developed cardiovascular collapse and shock within a few hours following ingestion of 50 mL chromic

acid (Loubieres *et al.*, 1999; ATSDR, 2008). A woman ingested 400 ml of leather tanning solution containing 48 grams of basic chromium sulphate ($CrOHSO_4$) and died of cardiogenic shock, complicated by pancreatitis and gut mucosal necrosis and haemorrhage (van Heerden *et al.*, 1994; ATSDR, 2008). Reports also revealed that a 33year old male developed hypotension, ventricular arrhythmias, severe respiratory distress and metobolic acidosis after ingesting an unknown amount of a liquid wood preservative that contains chromium trioxide, arsenic pentoxide, and copper oxide (Hay *et al.*, 2000, ATSDR, 2008).

2.4.5.8 Reproductive and Developmental Effects

One study showed wives of stainless steel welders were at higher risk of spontaneous abortions (Bonde *et al.*, 1992; ATSDR, 2008). The more recent study (Hjollund *et al.* 1995; ATSDR, 2008), however, did not corroborate those findings. No data were located regarding chromium in adverse human developmental effects. Several animal studies provide evidence that Chromium VI, after oral exposure, is a developmental toxicant in rats and mice (ATSDR, 2000; ATSDR, 2008).

Adverse developmental effects in animals include greater incidence of post-implantation loss, decreased fetal body weight, reduced ossification, and decreased number of live foetuses (ATSDR, 2008).

2.4.5.9 Genotoxic and Mutagenic Effects

The mechanism of chromium-induced genotoxicity is not fully understood. In one experiment, Chromium VI plus glutathione induced DNA damage in vitro, whereas Chromium III with or without glutathione did not. Chromium seems to exert its genetic effects by binding directly to DNA. It can produce stable DNA-chromium complexes, DNA strand breaks, DNA-DNA cross links, and DNA-protein cross links. The active species for

DNA binding seems to be the trivalent form (De Flora *et al.*, 1990, Cohen *et al.*, 1993; Meditext, 2005; ATSDR, 2008).

A recent clinical study reported strong DNA oxidative damage from the urinary samples of the patient who ingested 2 to 3 grams of potassium dichromate in a suicide attempt (Hanison *et al.*, 2005). Another study showed an involvement of the oxidative damage pathway in the mechanism of toxicity of chromium in occupationally exposed individuals (Goulart *et al.*, 2005; ATSDR, 2008).

Chromium VI compounds are clearly mutagenic in the majority of experimental situations (De Flora *et al.* 1990; Cohen *et al.*, 1993; ATSDR, 2008). It has caused chromosome aberrations in mammalian cells and has been associated with increased frequencies of chromosome aberrations in lymphocytes from chromate production workers. Increases in sister chromatid exchanges were seen in lymphocytes from workers exposed to chromium, cobalt, and nickel dusts (WHO, 1990; Meditext, 2005; ATSDR, 2008).

2.4.5.10 Carcinogenic Effects

Occupational exposure to Chromium VI compounds in a number of industries has been associated with increased risk of respiratory system cancers (ATSDR, 2000; ATSDR, 2008).

Baetjer was one of the first to review the literature prescated prior to 1950 on the occurrence of cancer in chromate-exposed workers (Baetjer, 1959b; ATSDR, 2008). The first epidemiological study of chromate production workers in the United States that demonstrated an association with lung cancer was conducted with 1,445 workers in seven plants engaged in the extraction of chromates from ore from 1930 to 1947. The percentage death due to cancer of the respiratory system was 21.8%; the percentage expected was 1.4% (Machle and Gregorius, 1948; ATSDR, 2008).

Studies of workers in the chromium pigment, chrome-plating, and ferrochromium industries showed a statistically significant association between worker exposure to Chromium VI and lung cancer (Langard and Norseth, 1975; Sheffet *et al.*, 1982; Frentzel-Beyme, 1983; Langard and Vigander, 1983; Davies, 1984; ATSDR 2000; ATSDR, 2008).

In addition to lung cancer, a number of epidemiological studies of workers in chromate industries also showed significantly increased risk for nasal and sinus cancers (ATSDR, 2000; ATSDR, 2008).

On the basis of these and other studies, the U.S. Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) have classified inhaled Chromium (VI) as a known human carcinogen (IARC, 1990; EPA, 1998). The World Health Organization (WHO) has determined that Chromium VI is a human carcinogen. The Department of Health and Human Services (DHHS) has determined that Chromium (VI) compounds are known to cause cancer in humans (ATSDR, 2000; ATSDR, 2008).

Carcinogenicity appears to be associated with the inhalation of the less soluble/insoluble Chromium (VI) compounds. A number of chronic inhalation studies provide evidence that Chromium VI is carcinogenic in animals (ATSDR, 2000; ATSDR, 2008) and no evidence exists to indicate that Chromium III can cause cancer in animals or humans (IARC 1990; EPA 1998; ATSDR, 2008).

2.4.5.11 Other Effects

Symptoms such as dizziness, headache, and weakness can be observed in workers working with a chrome plating plant with poor exhaust due to excessive high concentration of chromium trioxide fumes (Lieberman, 1911; ATSDR, 2008).

Chromium VI compounds exposure can cause erosion and discolouration of the teeth. Papillomas of the oral cavity and larynx have been reported in workers exposed to high air concentration of Chromium VI (Hathaway *et al*., 1996; ATSDR, 2008).

Severe corneal injury may result from ocular contact with solid or concentrated solutions of chromic acid and other Chromium VI compounds (Grant, 1993; ATSDR, 2008).

2.5 ANTIOXIDANTS

Antioxidants are molecules that inhibit the oxidation of other molecules. They are reducing agents, and limit oxidative damage to biological structures (Sies, 1997). They are phytochemicals, vitamins and other nutrients that protect our cells from damage caused by free radicals. They act as radical scavengers, inhibit lipid peroxidation and other free radical-mediated processes, and therefore they protect the human body from several diseases attributed to the reactions of radicals (Repeno and Llesuy, 2002). Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

Antioxidants are classified into two which are hydrophilic and hydrophobic. The water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation (Sies, 1997). The human body has an elaborate antioxidant defense system and antioxidants can be manufactured within the body and also be extracted from food such as fruits, vegetables, seeds, nuts, meats, and oil (Vertuani *et al*., 2004). The different types of antioxidants include vitamin C, vitamin E, beta-carotene, lutein, lycopene, selenium, glutathione as well as some enzymes such as superoxide dismutase (SOD), catalase and various peroxidases (Dekkers *et al*., 1996).

2.5.1 ASCORBIC ACID (VITAMIN C)

Ascorbic acid also known as vitamin C is a monosaccharide oxidation-reduction (redox) catalyst found in both animals and plants. Humans can't produce ascorbic acid because one of the enzymes needed to make ascorbic acid has been lost by mutation during primate evolution; therefore it must be obtain from the diet (Smirnoff, 2001). Most other animals are able to produce ascorbic acid in their bodies and do not require it in their diets (Linster and Van Schaftingen, 2007). Ascorbic acid is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species (ROS) such as hydrogen peroxide (Padayatty *et al*., 2003). In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants (Shigeoka *et al*., 2002). Ascorbic acid is present at high levels in all parts of plants and can reach concentrations of 20 millimolar in chloroplasts (Smirnoff and Wheeler, 2000).

2.5.2 VITAMIN E

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols. They are fat-soluble vitamins with antioxidant properties (Herrera and Barbas, 2001; Packer *et al*., 2001). α-tocopherol is considered to have the highest bioavailability amidst them with the body preferentially absorbing and metabolising this form (Brigelius-Flohé and Traber, 1999). It is also claimed to be the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Herrera and Barbas, 2001; Traber and Atkinson, 2007). This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidised α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol (Wang and Quinn, 1999). This is in line with findings showing that α-tocopherol, but not water-

soluble antioxidants efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death (Seiler *et al.*, 2008). GPx4 is the only known enzyme that efficiently reduces lipid-hydroperoxides within biological membranes.

2.5.3 GLUTATHIONE

Glutathione is a cysteine-containing peptide found in most forms of aerobic life (Meister and Anderson, 1983). It is synthesized in cells from its constituent amino acids (Meister, 1988). Glutathione has antioxidant properties this is because the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. Glutathione is maintained in the reduced form by the enzyme glutathione reductase in cells and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants (Meister, 1994). Glutathione is one of the most important cellular antioxidants and this is due to its high concentration and central role in maintaining the cell's redox state (Meister and Anderson, 1983).

2.6 CATALASE

Catalase is an enzyme found in nearly all living organisms exposed to oxygen. It is one of the most potent catalysts known and the reactions it catalyses are very crucial to life. It catalyzes the decomposition of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Zámocký and Koller, 1999; Chelikani *et al.*, 2004).

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Catalase protects cell from oxidative damage by reactive oxygen species (ROS). It also has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second (Goodsell,

2007). In Eukaryotic cells, Catalase is usually located in a cellular organelle called the peroxisome (del Rio *et al*., 1992; Alberts *et al*., 2002). The optimum pH for human catalase is approximately 7 (Machly and Chance, 1954). The optimum temperature also varies by species (Toner *et al*., 2007). Human catalase works at an optimum temperature of 37°C (Aebi, 1984), which is approximately the temperature of the human body.

In determining the catalase activity of tissue homogenates, dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate.

2.7 SUPEROXIDE DISMUTASE (SOD)

Superoxide dismutases (SODs) are enzymes that catalyze the breakdown of superoxide anion into oxygen and hydrogen peroxide (Bannister *et al*., 1987; Zelko *et al*., 2002). Superoxide dismutase (SOD) enzymes are present in almost all aerobic cells and in extracellular fluids (Johnson and Giulivi, 2005). They are an important antioxidant defence in nearly all cells exposed to oxygen. They contain metal ion cofactors and this depends on the isozyme which can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion (Bannister *et al*., 1987). A third form of SOD also exists in extracellular fluids and it contains copper and zinc in its active sites (Nozik-Grayck *et al*., 2005). The mitochondrial isozyme seems to be the most biologically important of these three, since mice lacking this enzyme die soon after birth (Melov *et al*., 1998).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

- Weighing balance
- Dissecting set
- Dissecting board
- Conical flasks
- Beakers
- Homogenizer
- Cold centrifuge
- Sample bottles
- Hand gloves
- Cotton wool
- Oral cannula
- Syringes and needles
- Spectrophotometer
- Atomic Absorption Spectrophotometer (AAS)
- Micropipette
- Thread
- Chromic catgut

3.2 CHEMICALS

- Chromium trioxide (CrO_3) (Acros Chemicals, New Jersey)
- Chromium (III) oxide (Cr_2O_3) (Kermel Chemicals, China)
- Indomethacin
- Sodium bicarbonate
- Formalin
- Xylazine
- Ketamine
- Sodium Phosphate monobasic anhydrous (Na_2HPO_4) (BDH Chemicals, England)
- Sodium Phosphate dibasic anhydrous (NaH_2PO_4) (BDH Chemicals, England)
- Stock Bovine Serum Albumin (Standard) (Sigma Chemical Co., USA)
- Copper Sulfate ($CuSO_4.5H_2O$) (BDH Chemicals, England)
- Sodium Tartarate (BDH Chemicals, England)
- Potassium Iodide (KI) (BDH Chemicals, England)
- Trichloroacetic acid (TCA) (Oxford Laboratory reagent, India)
- Thiobarbituric acid (TBA) (BDH Chemicals, England)
- Tris base (BDH Chemicals, England)
- Potassium Chloride (KCL) (BDH Chemicals, England)

3.3 EXPERIMENTAL ANIMALS

Sixty (60) male albino rats of the wistar strain weighing between 100 and 120g were used for this study. The animals were housed in the animal house of the College of Medicine, University of Ibadan and they were acclimatized for two weeks and fed with rat feed and given water ad libitum.

3.4 ANIMAL GROUPS

The animals were divided into six groups as follows.

Table 1: Animal Grouping

Group	Name	Description
1	Non-ulcerated control (nCont)	Received tap water
2	Ulcerated control (uCont)	Received tap water
3	Chromium III at 10ppm (ChrIII-10)	Received water containing 10ppm concentration of Chromium (III)
4	Chromium III at 100ppm (ChrIII-100)	Received water containing 100ppm concentration of Chromium (III)
5	Chromium VI at 10ppm (ChrVI-10)	Received water containing 10ppm concentration of Chromium (VI)
6	Chromium VI at 100ppm (ChrVI-100)	Received water containing 100ppm concentration of Chromium (VI)

3.5 PROCEDURES

Animals were exposed to Chromium (VI) in the form of Chromium trioxide (CrO_3) and Chromium (III) in the form of Chromium (III) oxide (Cr_2O_3) in drinking water for 12 weeks and fed with commercial rat pellets.

3.6 BLOOD CHROMIUM CONCENTRATION DETERMINATION

Blood was collected from the animals via cardiac puncture at the end of the 12 weeks exposure to Chromium. 1ml of the blood collected was transferred into a test tube and the blood was digested with 2ml of nitric acid (HNO_3) and left overnight. The digested blood was placed in water bath and heated for 30 minutes at 100°C. However, after cooling down, 12ml of distilled water was added to the digested blood and was filtered. The Chromium concentration of the filtrate was read using Atomic Absorption Spectrophotometer (AAS) (Marouani *et al*., 2012).

3.7 INDUCTION OF GASTRIC ULCER

Two different gastric ulcer models were used for this study and these are;

- Indomethacin-induced ulcer
- Pylorus ligation induced ulcer

3.7.1 INDOMETHACIN- INDUCED ULCER

The animals were fasted for 24hours before administration of Indomethacin (Shirsha and Subash, 2012). Ulcer was induced in the animals with indomethacin at a dose of 40 mg/kg body weight. The animals were sacrificed 4hours later after which the stomach was removed and opened along the greater curvature and ulcers formed were scored according to the method of Shirsha and Subash. (2012).

3.7.2 PYLORUS LIGATION INDUCED ULCER

Under Ketamine and Xylazine anaesthesia, the abdomen was opened and the pylorus end of stomach was ligated and replaced back carefully. The abdomen was sutured and animals were sacrificed 4 hours after ligation of Pylorus. The stomachs were removed and the gastric

contents were drained into tubes and were centrifuged at 1000 rpm for 10 minutes and the volume of the supernatant was noted. The stomachs were rinsed and severity of ulcer formed were assessed and scored according to the method described by Shirisha and Subash. 2012.

Table 2: Scoring system for ulceration (Rasika *et al.*, 2010; Shirisha and Subash, 2012).

Score	Criteria
0	Normal stomach
0.5	Red colouration
1	Spot ulcers
1.5	Haemorrhagic streaks
2	Ulcers >3mm but < 5mm
3	Ulcers > 5mm

3.8 DETERMINATION OF GASTRIC ACIDITY

0.5ml of the gastric juice was pipetted into 100ml conical flask and 2 drops of phenolphthalein solution was added and was titrated with 0.01N sodium hydroxide (NaOH). The volume of alkali added was noted. The volume corresponds to total acidity.

The acidity was calculated by using the formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \times \text{mEq/100gm}$$

3.9 ORGAN COLLECTION AND PREPARATION OF TISSUE FOR BIOCHEMICAL ASSAY AND HISTOLOGICAL OBSERVATION

The animals were sacrificed by cervical dislocation four hours after induction of ulcer in both models and the abdominal cavity was opened to remove the stomach. The stomachs were opened along the greater curvature, rinsed in ice-cold phosphate buffer saline after which they were blotted and weighed. The stomachs were then minced with scissors into two sections, a section stored in 6 volumes of ice-cold 0.1M phosphate buffer, pH 7.4, and homogenized using homogenizer. The resulting homogenates were centrifuged at a speed of 10,000rpm 4°C for 10minutes. The supernatant – post mitochondrial fraction (PMF) were collected and processed for biochemical estimations (Hussain *et al.*, 2009). The second section was fixed in 10% buffered formalin for histological assessment.

3.10 ASSESSMENT OF LIPID PEROXIDATION

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale (1970).

Principle

Under acidic condition, malondialdehyde (MDA) produced from the peroxidation of fatty acid membranes and food products react with the chromogenic reagent, 2-thiobarbituric acid (TBA) to yield a pink coloured complex with maximum absorbance at 532nm and fluorescence at 553nm. The pink chromophore is readily extractable into organic solvents such as butanol.

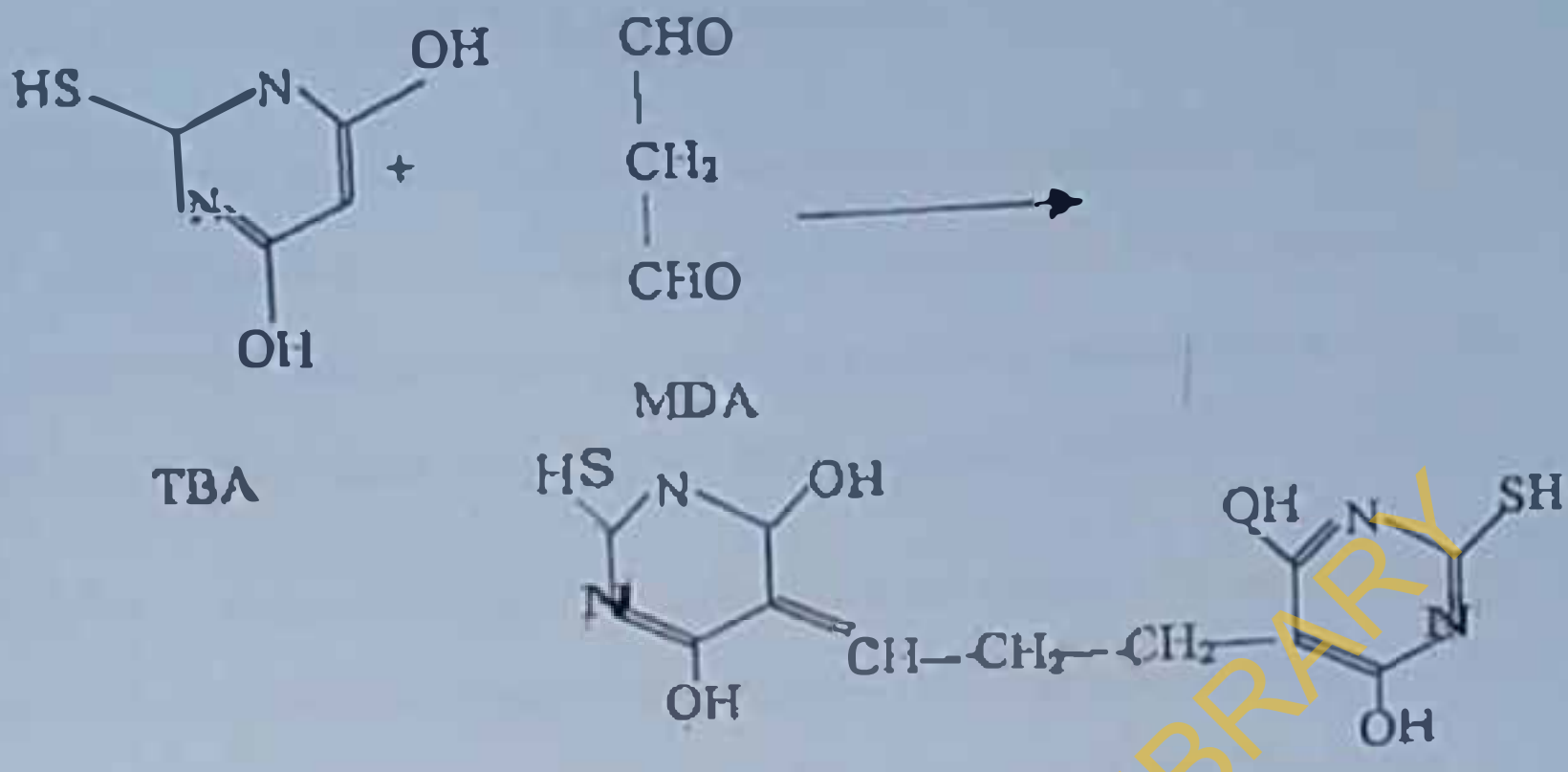


Figure 4: Reaction of TBA with MDA

Procedure

1.6ml of Tris-KCl buffer was mixed with an aliquot of 0.4ml of the test sample (i.e Post mitochondrial supernatant) to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. This was then cooled in ice and centrifuged at 3000rpm. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532nm. The MDA level was calculated according to the method of Adam-Vizi and Seregi (1982). Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}Cm^{-1}$.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance} \times \text{Volume of mixture}}{E_{532nm} \times \text{volume of sample} \times \text{mg protein}}$$

3.11 DETERMINATION OF CATALASE ACTIVITY

Catalase activity was determined according to the method of Sinha (1972). This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced is measured spectrophotometrically at 570nm.

The presence of dichromate in the assay mixture does not interfere with the colorimetric determination of chromic acetate because it does not have any absorbency in the region. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate/acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Determination of Catalase activity of samples

0.1ml of sample was mixed with 0.4ml of distilled water to give 1 in 5 dilution of the sample. The assay mixture contained 2ml of hydrogen peroxide (H_2O_2) solution (400μmoles) and 2.5ml of phosphate buffer in a 10ml flat bottom flask. The reaction was run at room temperature. A 1ml portion of the reaction mixture was withdrawn and blown into 1ml dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide contents of the withdrawn sample were determined by the method described above.

3.12 DETERMINATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

The activity profile of SOD in the homogenates was determined by the method of Misra and Fridovich (1972).

Principle

The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase.

Superoxide ($O^{\cdot}_2$) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per $O^{\cdot}_2$ introduced increased with increasing pH (Valerino and Mc Cormick, 1971) and also increased with increasing concentration of epinephrine. These results led to the proposal that autoxidation of epinephrine proceeds by at least two distinct pathways, only one of which is a free radical chain reaction involving superoxide ($O^{\cdot}_2$) radical and hence inhibitable by SOD.

Procedure

1ml of sample was diluted in 9ml of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

CALCULATION: Increase in absorbance per minute = $\frac{A_1 - A_0}{2.5}$

Where, A_0 = absorbance after seconds

A_1 = absorbance 150 seconds

% inhibition = $\frac{\text{Increase in absorbance for substrate} \times 100}{\text{Increase in absorbance of blank}}$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.

3.13 HISTOLOGICAL ANALYSIS

Step 1 (Collection of Tissues): The stomach was removed and it was opened along the greater curvature and rinsed in normal saline to remove the food debris.

Step 2 (Fixation of Tissues): The stomach then was placed in sample bottle containing 10% formalin.

Step 3 (Dehydration): Water was removed from the tissue by putting it in ascending grades of alcohol (70%, 80%, 90%, 2 changes of 100%) one hour each. Ascending concentrations of alcohol was used to prevent sudden rush out of water from the tissues, so that the cell will not be distorted or damaged.

Step 4 (Clearing): Alcohol was then removed from the tissues because it is not miscible with paraffin. Xylene also made the opaque tissue transparent, therefore the name clearing stage. The tissue was passed twice through xylene, and spent 2 hours each time.

Stage 5 (Embedding): The tissue was infiltrated and impregnated in 2 changes molten paraffin wax one hour each. It was allowed to cool on a frozen surface then removed.

Step 6 (Microtomy): The tissue was trimmed to expose tissue surface with microtome, and was cooled on ice. 5μ of tissue was sectioned.

Step 7 (Mounting of Paraffin Sections): Float using 2% alcohol into a warm water of about 2°C below melting point of wax. Use clean, grease free slide to pick the floating section. The other side of the slide was cleaned and placed on hot plate after proper labelling for about 3 hours for the section to be completely fixed and the slide to dry.

Step 8 (Staining): The section was deparaffinised in 2 changes of xylene for 1 minutes each so that the stains can permeate. The slide was then immersed in a descending concentration of alcohol (i.e 100%, 90%, 80%, and 70%) for 1 minute in each alcohol solution so as to dehydrate it. The slides were rinsed in water and placed in Ehlich heamatoxylin for about 15 minutes The slides were dipped in 1% acid-alcohol (2 dips) and rinsed in running water for about 3 minutes till the colour of the section to become blue. The slides were immersed in ascending grades of alcohol (70%, 80%, 90% and 100%) for about 30 seconds so as to dehydrate the preparation. The preparation was cleared of alcohol by dipping it in xylene for 1minute After these, the slide was blotted and mounted under a cover slip using dibutylphthalene xylene (DPX), and air bubbles were prevented from getting in. the slide was then read under the microscope using x100 magnification and lesions were noted. A photomicrograph of the slide was then taken. The extent of tissue injury was scored on a scale of tissues contraction, regeneration of the ulcerated mucosa, and inflammatory exudates was observed under stereomicioscope

3.14 STATISTICAL ANALYSIS

Values were expressed as Mean±SEM and were analyzed using one way ANOVA and student t- test. Values were considered significant at $p<0.05$.

CHAPTER FOUR

RESULTS

4.1 CONCENTRATION OF CHROMIUM IN THE BLOOD OF EXPERIMENTAL RATS

Twelve week exposure of experimental rats to Chromium caused a significant increase in the blood chromium level of chromium treated groups (ChrIII-10: 0.12±0.01; ChrIII-100: 0.12±0.01; ChrVI-10: 0.13±0.01 and ChrVI-100: 0.22±0.03) when compared with the non-ulcerated control group (nCont) (0.08±0.01). This is illustrated in Figure 5.

Figure 5: Blood Chromium concentration at 12 weeks

* Significant at p= 0.05 when compared with the non-ulcerated control group (nCont)

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

ChrIII-100: Chromium III at 100ppm

ChrVI-10: Chromium VI at 10ppm

ChrVI-100: Chromium VI at 100ppm

4.2 EFFECT OF CHROMIUM ON PYLORIC LIGATION INDUCED ULCERATION

Ligation of the stomach pyloric end caused ulceration in the rats' stomach. The mean ulcer score of chromium exposed groups (ChrIII-10: 1.63±0.24; ChrIII-100: 2.75±0.14; ChrVI-10: 1.75±0.25 and ChrVI-100: 2.75±0.25) were significantly lower compared with the ulcerated control (uCont) group (4.00±0.45) (Figure 6).

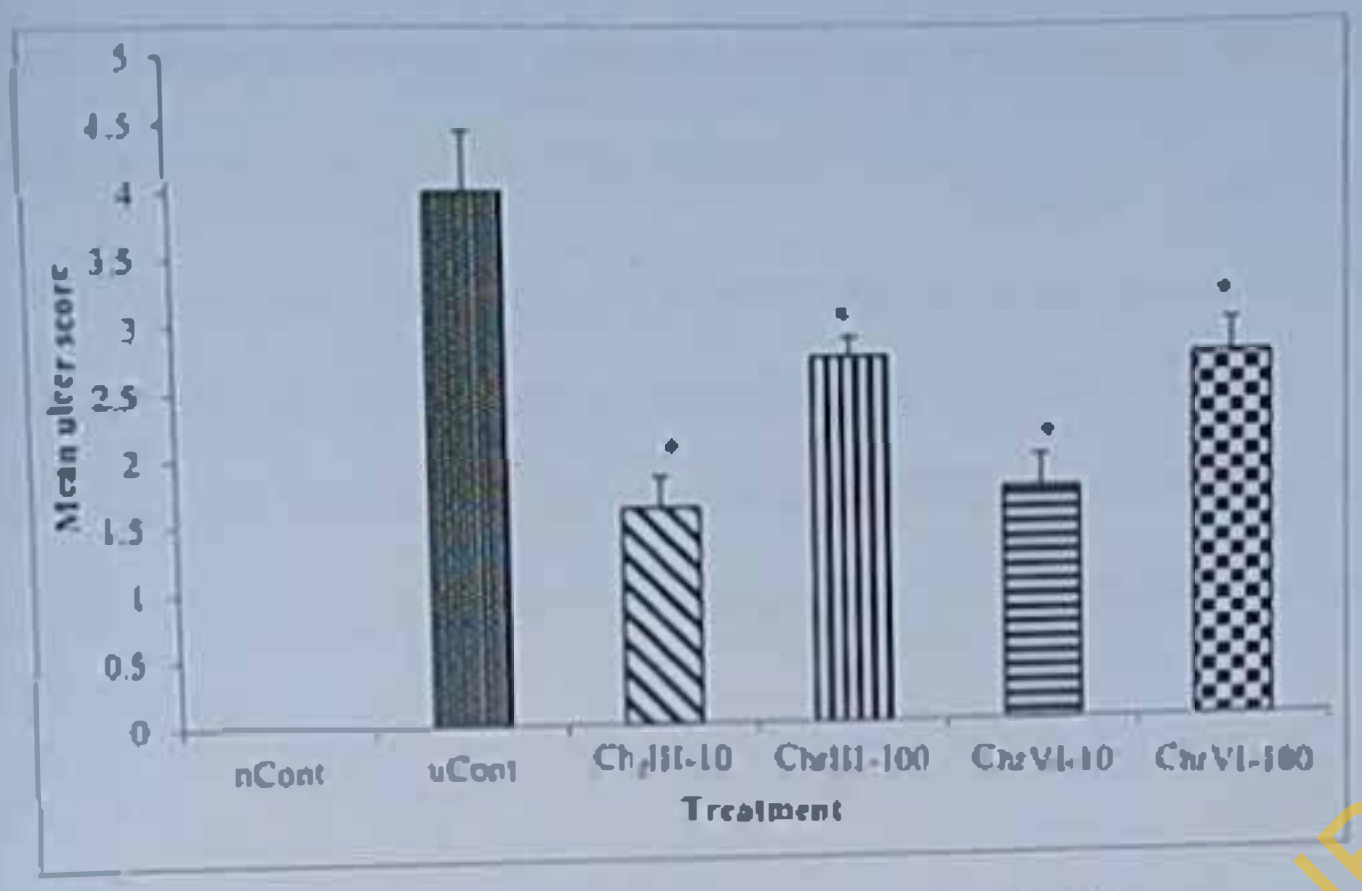


Figure 6. Effect of Chromium on mean ulcer score in pylorie ligation ulcer model

* Significnnt at p=0.05 when compared with the Ulcerated control (uCont)

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

ChrIII-100: Chromium III at 100ppm

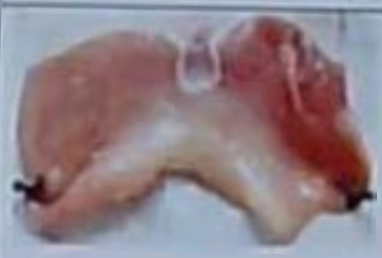
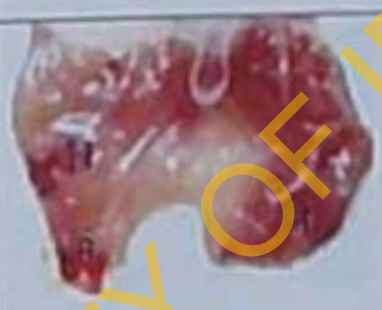
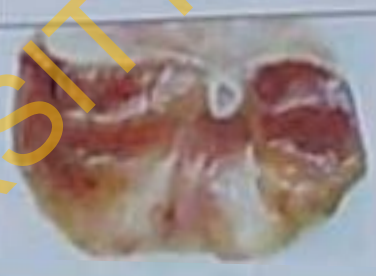
ChrVI-10: Chromium VI at 10ppm

ChrVI-100: Chromium VI at 100ppm

4.3 MACROSCOPIC APPEARANCE OF STOMACH TISSUE IN PYLORIC LIGATION INDUCED ULCER MODEL

The non-ulcerated control (nCont) stomach appears normal, there is no visible lesion. Thus, the ulcerated control stomach is characterized by ulcer which is greater than 3mm but less than 5mm in diameter. The chromium exposed groups are distinguished by red colouration haemorrhagic streaks and ulcers greater than 3mm but less than 5mm in diameter. This can be seen in Table 3 below.

Table 3: Mean Ulcer score and macroscopic appearance of stomach tissue in pyloric ligation ulcer model

Treatment groups	Macroscopic appearance	Mean Ulcer Score
nCont		0.00±0.00
uCont		3.00±0.45
ChrIII-10		1.63±0.24*
ChrIII-100		2.75±0.14*
ChrVI-10		1.75±0.25*
ChrVI-100		2.75±0.25*

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

H: Haemorrhagic streak

R: Red colouration

U: Ulcers greater than 3mm < 5mm

4.4 EFFECT OF CHROMIUM ON GASTRIC ACID OUTPUT VOLUME IN PYLORIC LIGATION INDUCED ULCER MODEL

The volume of the gastric acid output was significantly lower in Chromium exposed groups (ChrIII-10: 0.55±0.04; ChrIII-100: 0.80±0.04; ChrVI-10: 0.45±0.07) compared with the ulcerated control (uCont) (1.33±0.22) group. The ChrVI-100 treated group (1.05±0.03) showed no significant difference when compared with ulcerated control (uCont) (Figure 7).

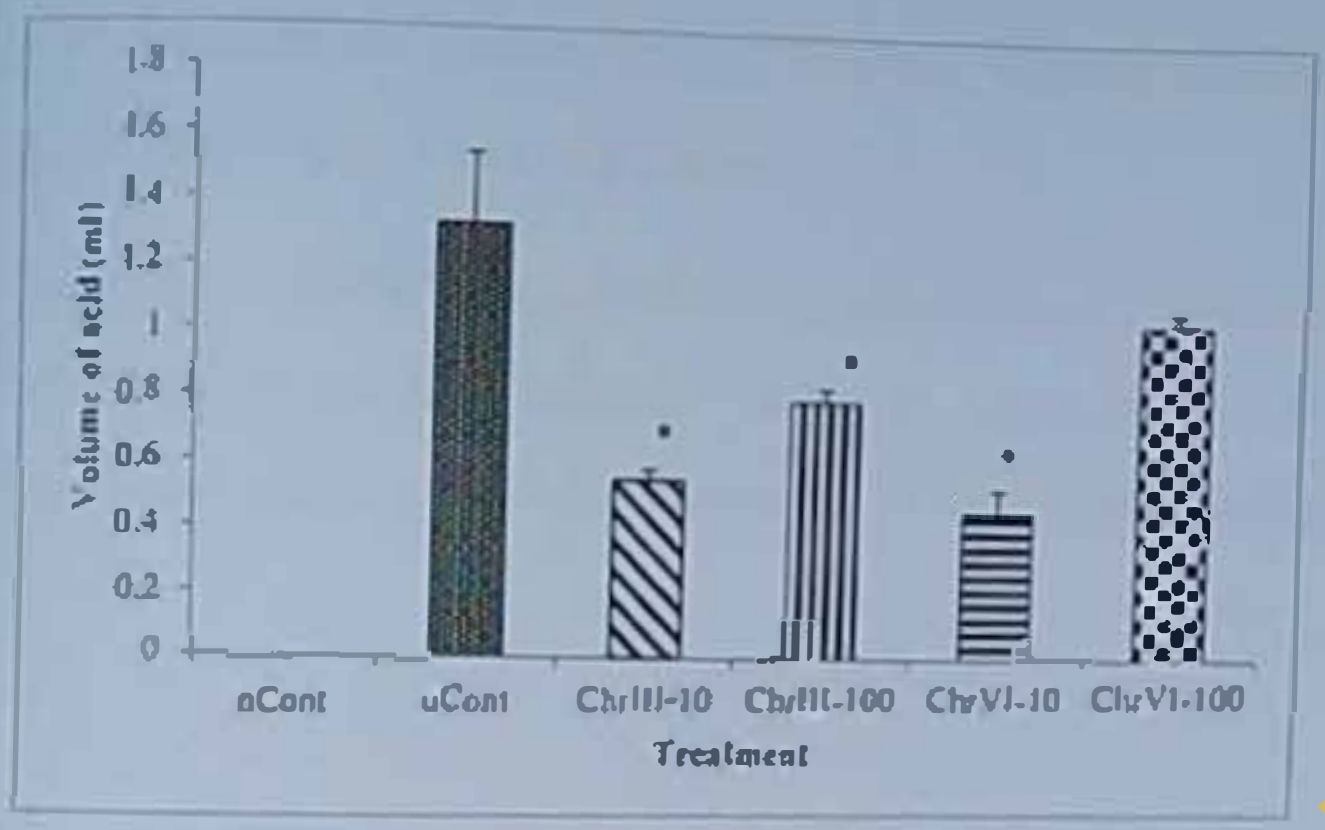


Figure 7: Effect of Chromium on Gastric acid volume in pyloric ligation ulcer model

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

ChrIII-100: Chromium III at 100ppm

ChrVI-10: Chromium VI at 10ppm

ChrVI-100: Chromium VI at 100ppm

4.5 EFFECT OF CHROMIUM ON GASTRIC OUTPUT ACIDITY IN PYLORIC LIGATION INDUCED ULCERATION

The acidity of gastric juice secreted was significantly lower in chromium exposed groups (CbrIII-10: 12.00±1.00; CbrIII-100: 26.92±2.47; CbrVI-10: 16.00±1.53; CbrVI-100: 26.75±1.46) compared with the ulcerated control (uCont) (34.00±1.00) (Figure 8).

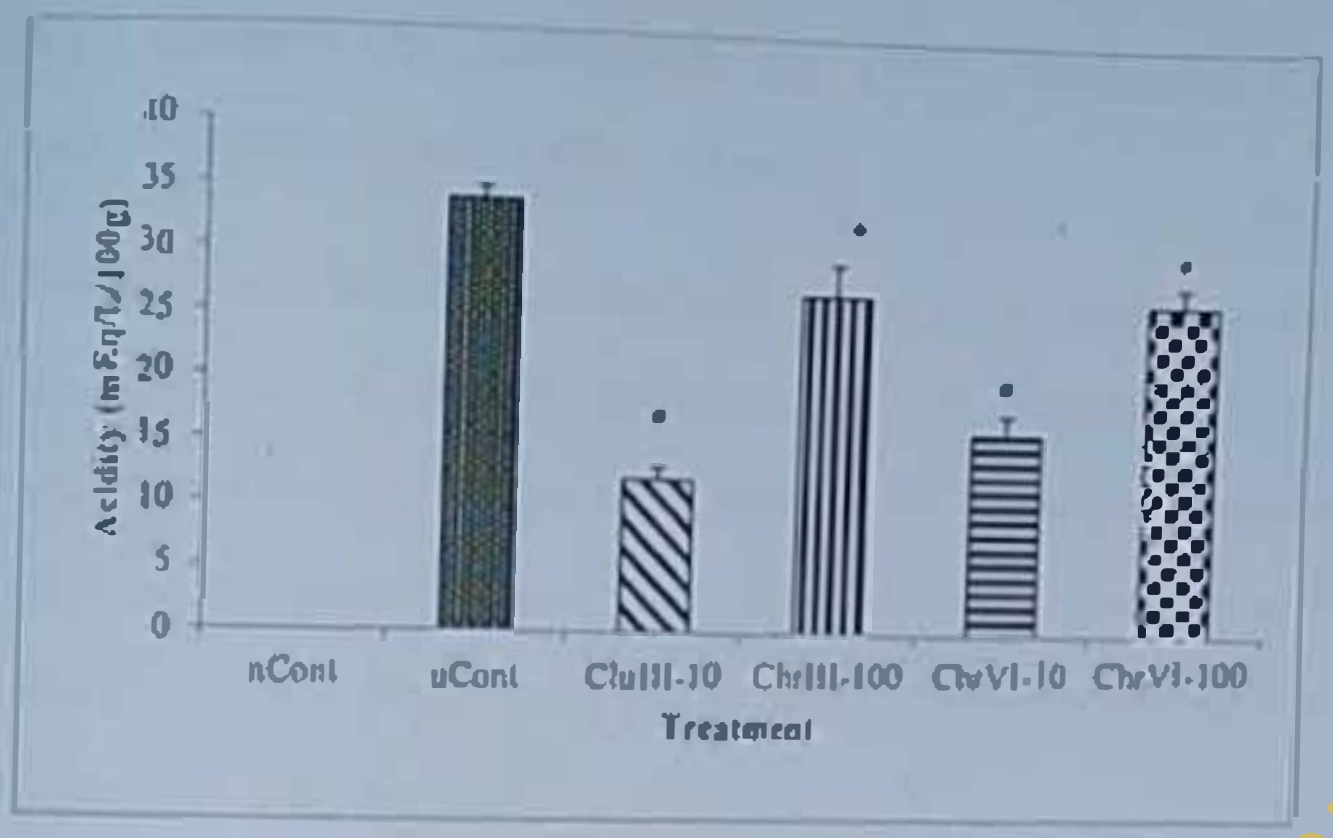


Figure 8: Effect of Chromium on Gastric output acidity in pyloric ligation ulcer model

* Significant at p>0.05 when compared with the Ulcerated control (uCont)

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

ChrIII-100: Chromium III at 100ppm

ChrVI-10: Chromium VI at 10ppm

ChrVI-100: Chromium VI at 100ppm

4.6 EFFECT OF CHROMIUM ON LIPID PEROXIDATION IN PYLORIC LIGATION INDUCED ULCERATION

The induction of ulcer caused a significant increase in lipid peroxidation level of all treatment groups (uCont: 11.87±0.07; ChrIII-10: 8.19±0.08; ChrIII-100: 8.95±0.28; ChrVI-10: 8.90±0.29; ChrVI-100: 9.72±0.17) when compared with the non-ulcerated control (nCont) (3.24±0.13). However, the lipid peroxidation levels of all chromium exposed groups were significantly decreased compared with the ulcerated control (uCont) (11.87±0.07). This result is illustrated in Figure 9 below.

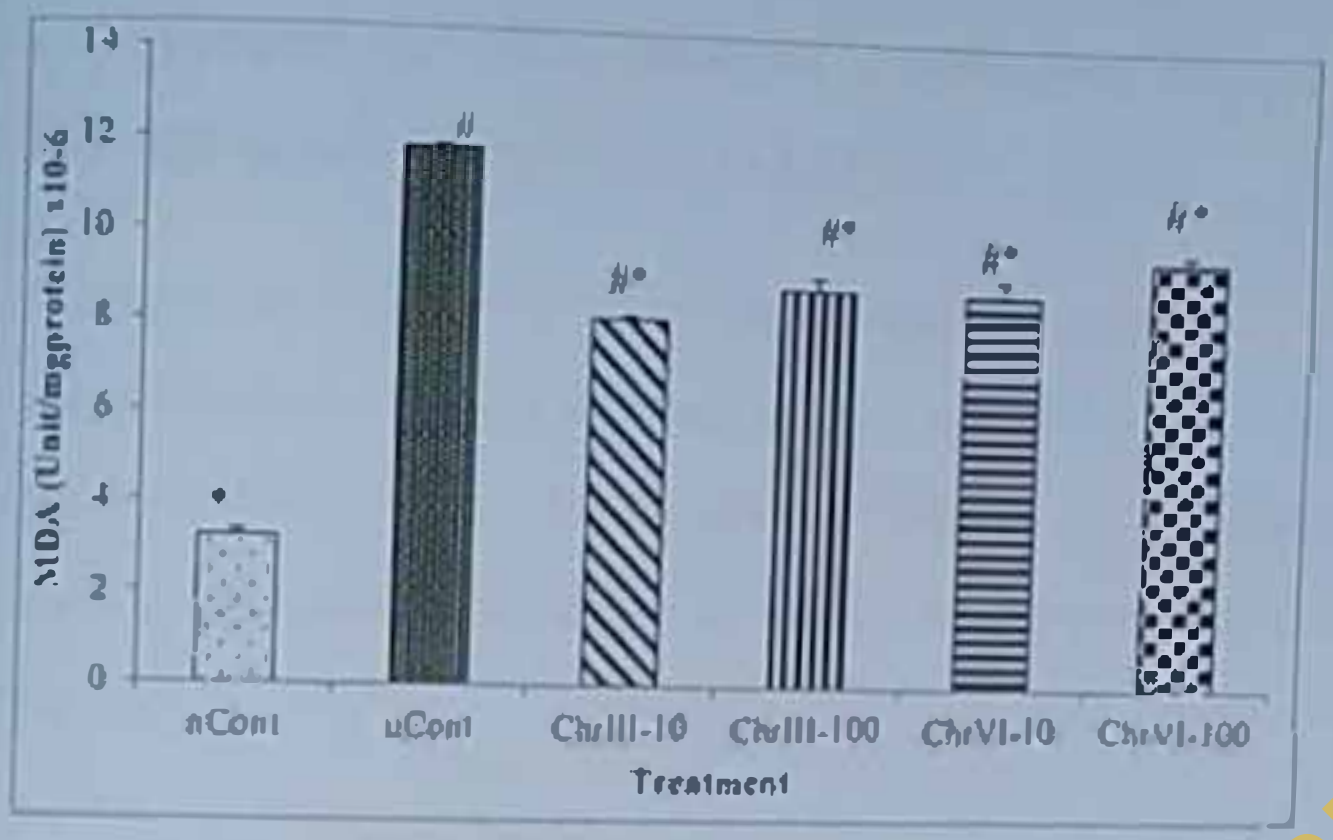


Figure 9: Effect of Chromium on lipid peroxidation in pyloric ligation ulcer model

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Significant at p=0.05 when compared with the non-ulcerated control (nCont)

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

ChrIII-100: Chromium III at 100ppm

ChrVI-10: Chromium VI at 10ppm

ChrVI-100: Chromium VI at 100ppm

4.7 EFFECT OF CHROMIUM ON SUPEROXIDE DISMUTASE (SOD) IN PYLORIC LIGATION INDUCED ULCERATION

The Superoxide Dismutase (SOD) activity was significantly higher in all chromium exposed groups (ChrIII-10: 115.34±1.35; ChrIII-100: 99.71±2.10; ChrVI-10: 106.72±0.88; ChrVI-100: 95.02±0.46) compared with the ulcerated control (uCont) (92.2±0.8) and significantly lower in all the groups compared with the non-ulcerated control (nCont) group (124.58±1.01) (Figure 10).

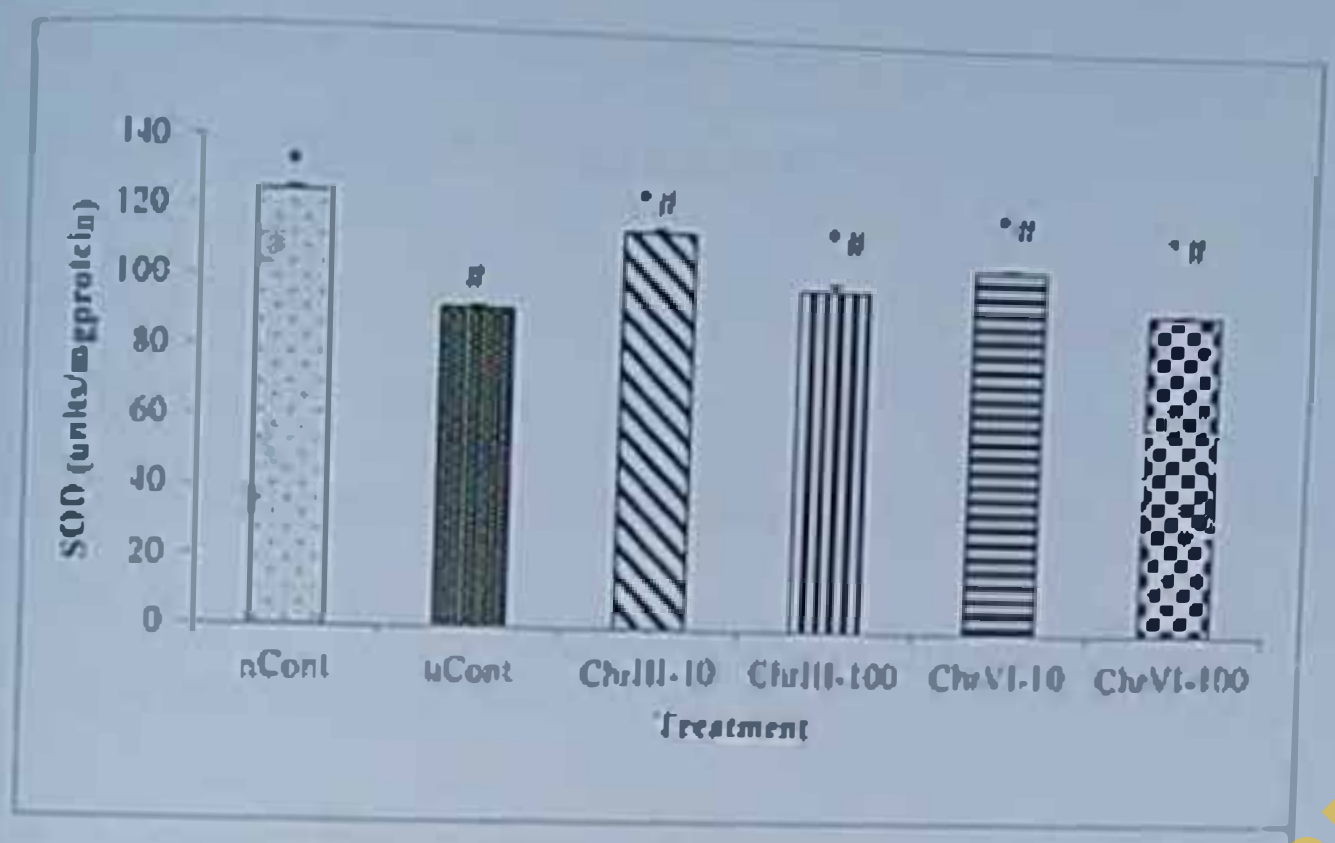


Figure 10: Effect of Chromium on Superoxide Dismutase (SOD) activity in pyloric ligation ulcer model

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Significant at p=0.05 when compared with the non-ulcerated control (nCont)

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

ChrIII-100: Chromium III at 100ppm

ChrVI-10: Chromium VI at 10ppm

ChrVI-100: Chromium VI at 100ppm

4.8 EFFECT OF CHROMIUM ON CATALASE ACTIVITY IN PYLORIC LIGATION INDUCED ULCER MODEL

The Catalase activity was significantly higher in all chromium exposed groups (ChrIII-10: 1173.95±10.92; ChrIII-100: 1021.25±13.68; ChrVI-10: 1089.10±7.20; ChrVI-100 974.42±6.82) compared with the ulcerated control (uCont) (930.74±9.28). They were however significantly lower compared to the non-ulcerated control (nCont) group (1508.01±20.22) (Figure 11).

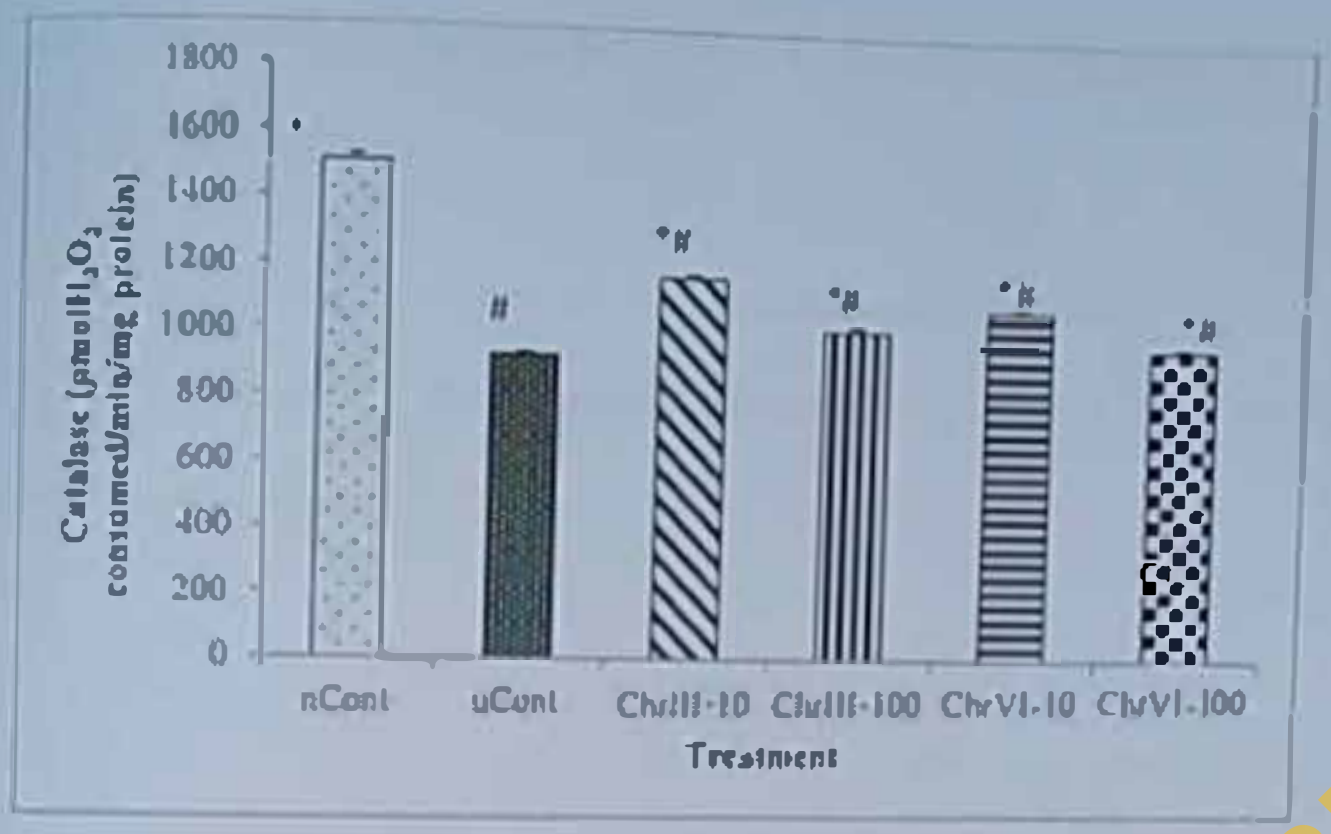


Figure 11: Effect of chromium on catalase activity in pyloric ligation ulcer model

* Significant at p=0.05 when compared with the Ulcerated control group

Significant at p=0.05 when compared with the non-ulcerated control group

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

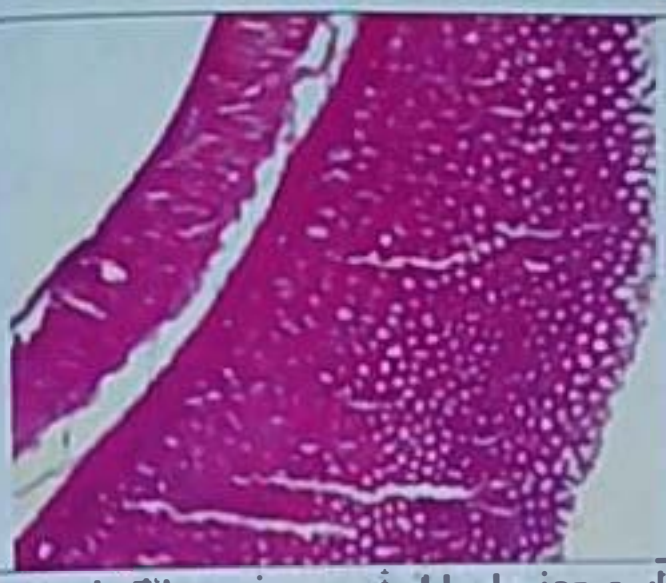
ChrIII-100: Chromium III at 100ppm

ChrVI-10: Chromium VI at 10ppm

ChrVI-100: Chromium VI at 100ppm

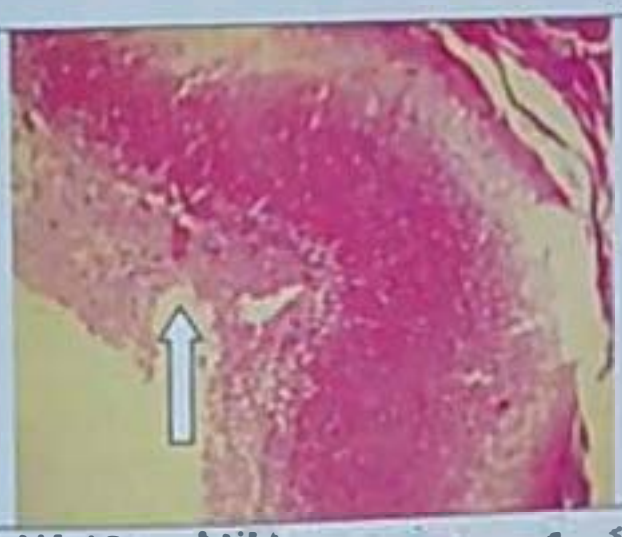
4.9 PHOTOMICROGRAPH OF STOMACH HISTOLOGY IN PYLORIC LIGATION MODEL

Histology showed erosion of the surface epithelium and marked congestion of blood vessels in the uCont group. The chromium exposed groups showed mild erosion of the surface epithelium as shown in Figure 12.



aControl: There is no visible lesion and the tunics are intact. H&E x100

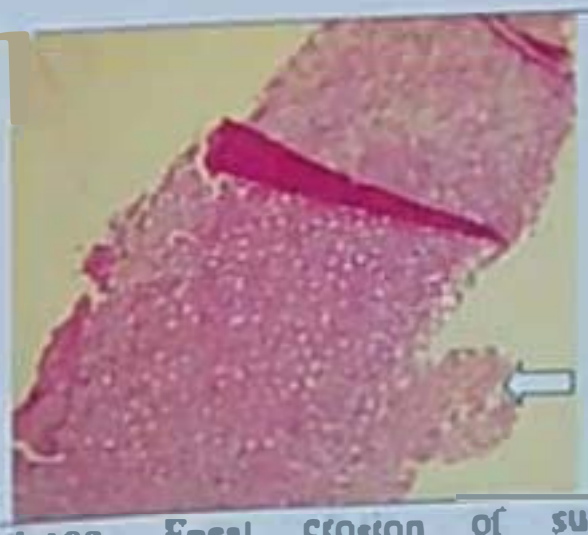
uControl: Marked congestion of submucosal blood vessels in squamous portion (black arrow), a few foci of erosion (white arrow) H&E x100



ChrIII-10: Mild erosion of surface epithelium (white arrow). H&E x100

ChrIII-100: Moderate congestion of blood vessels in tunic mucosa (white arrow). H&E x100

ChrVI-10: Moderate erosion of surface epithelium, marked congestion of blood vessels (white arrow). H&E x100



ChrVI-100: Focal erosion of surface epithelium and other tunics shows no visible lesion (white arrow). H&E x100

Figure 12: Photomicrographs of stomach tissues in pylorc ligation induced ulceration model

4.10 EFFECT OF CHROMIUM ON INDOMETHACIN INDUCED ULCERATION

Induction of ulcer using indomethacin caused significant ulceration in the ulcerated control (uCont) (12.60±0.48) compared to non-ulcerated stomach (nCont) (0.00±0.00). Chromium exposure decreases the degree of ulceration as seen in the chromium treated groups (ChrIII-10: 10.25±0.48; ChrIII-100: 10.78±0.18; ChrVI-10: 11.38±0.48; ChrVI-100: 12.10±0.56). This decrease was however significant only in the ChrIII-10 and ChrIII-100 groups (Figure 13).

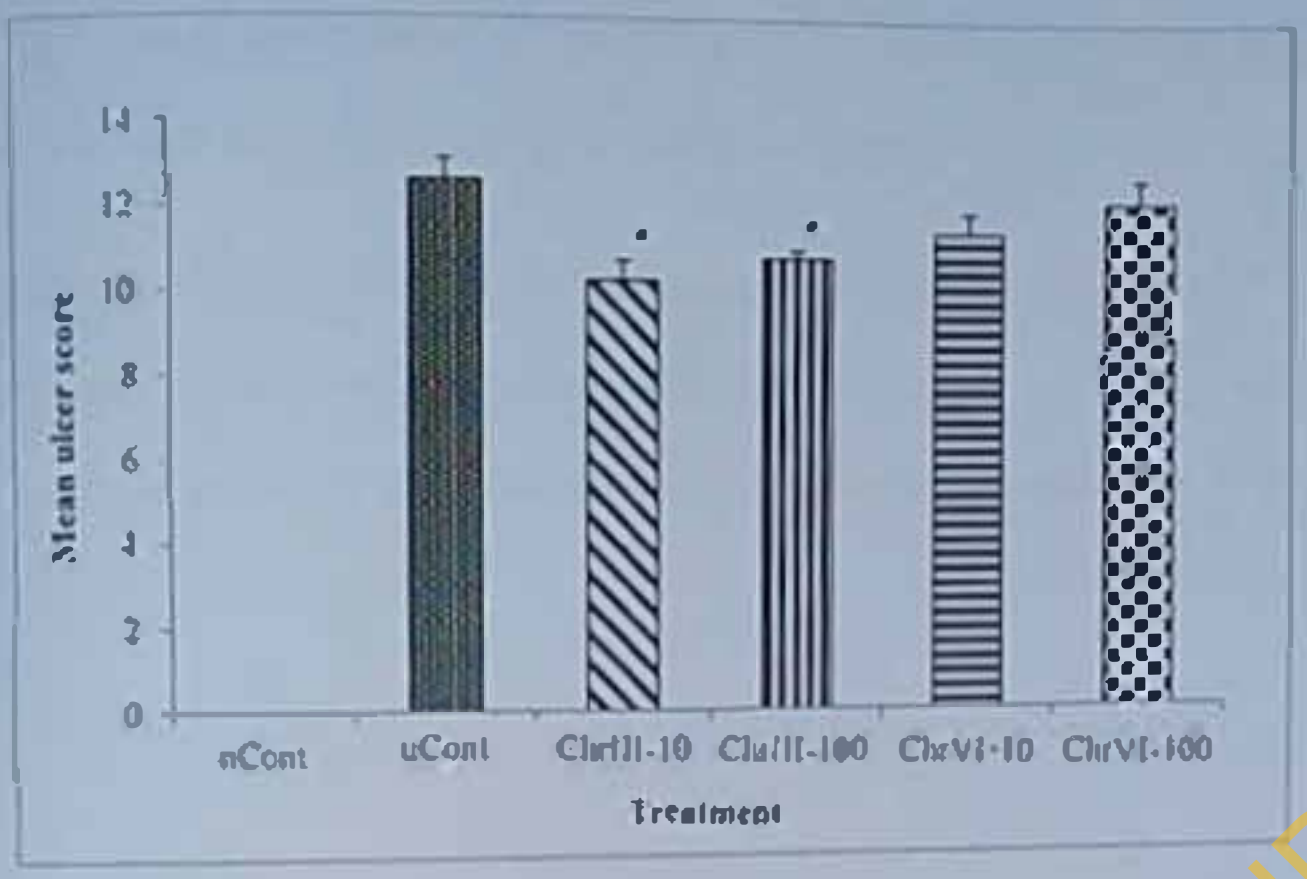


Figure 13: Effect of Chromium on Mean ulcer score in Indomethacin induced ulcer model

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

ChrIII-100: Chromium III at 100ppm

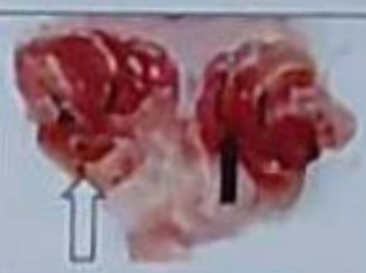
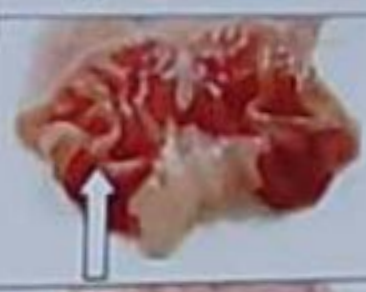
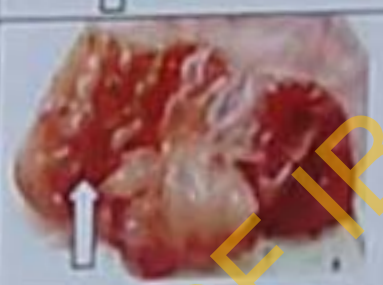
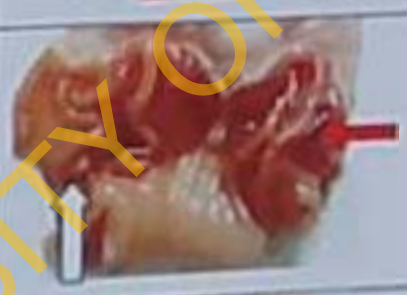
ChrVI-10: Chromium VI at 10ppm

ChrVI-100: Chromium VI at 100ppm

4.11 MACROSCOPIC APPEARANCE OF STOMACH TISSUE IN INDOMETHACIN INDUCED ULCER MODEL

There is no visible lesion in the non-ulcerated control (nCont) stomach. The ulcerated control (uCont) stomach has ulcers greater than 3mm in diameter and the chromium exposed groups are characterized by pin points or punctuate ulcers as shown in Table 4.

Table 4: Mean Ulcer score and macroscopic appearance of stomach tissue in indomethacin induced ulcer model

Treatment groups	Macroscopic appearance	Mean Ulcer score
nCont		0.00±0.00
uCont		12.60±0.48
ChrIII-10		10.25±0.48*
ChrIII-100		10.78±0.18*
ChrVI-10		11.38±0.17
ChrVI-100		12.10±0.56

* Significant at $p<0.05$ when compared with the Ulcerated control (uCont)

White Arrow: Pin point ulcers

Red Arrow: Two or more small haemorrhagic ulcers

Black Arrow: Ulcers greater than 3mm in diameter

4.12 EFFECT OF CHROMIUM ON LIPID PEROXIDATION IN INDOMETHACIN INDUCED ULCERATION

There was a significant increase in malondialdehyde value in uCont (4.34±0.06) when compared with non-ulcerated control nCont (3.21±0.03). However, all chromium exposed groups (ChIII-10: 4.88±0.03; ChIII-100: 5.29±0.11; ChV1-10: 5.58±0.04; ChV1-100: 5.96±0.03) significantly decreases MDA values in a dose dependent manner when compared with the ulcerated control (uCont) group (Figure 16).

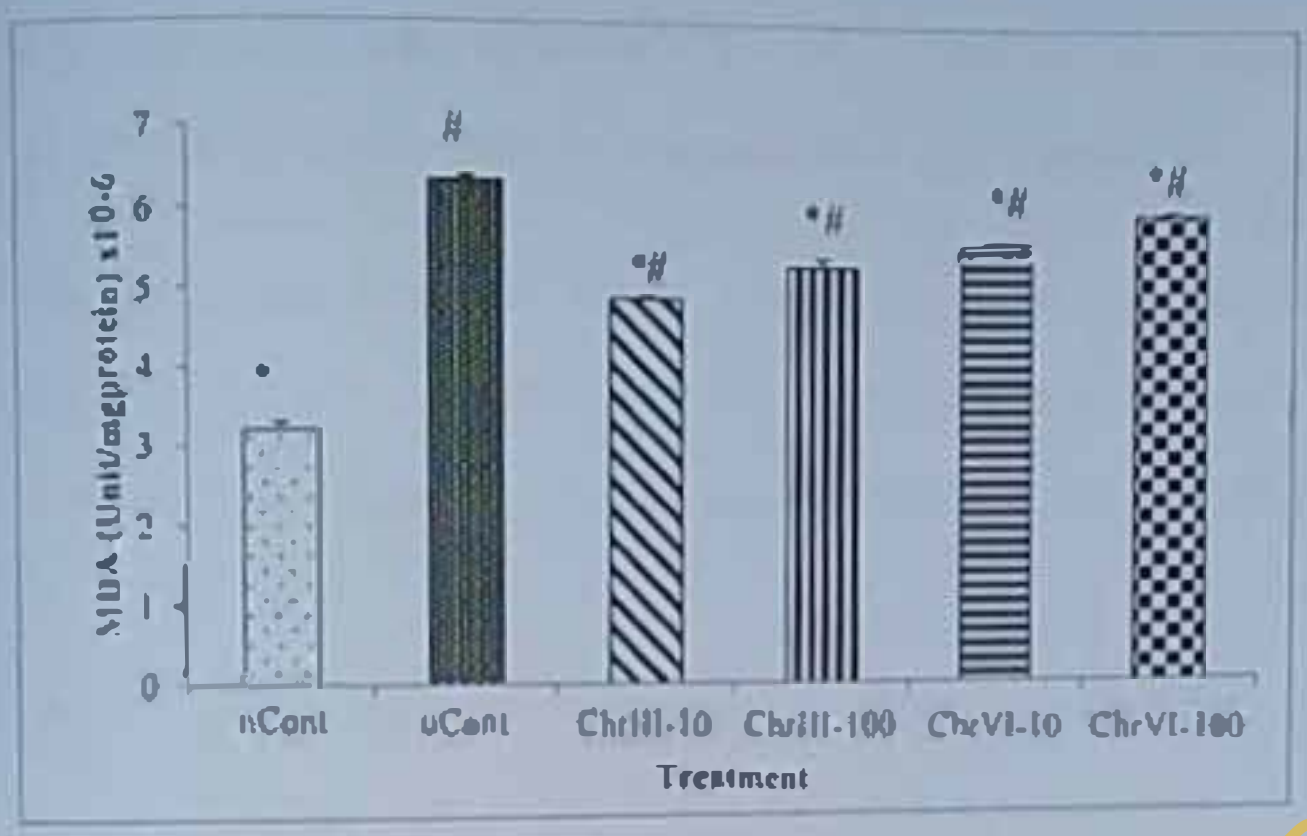


Figure 14: Effect of Chromium on Lipid peroxidation in indomethacin ulcer model

* Significant at p=0.05 when compared with the Ulcerated control group

Significant at p=0.05 when compared with the non-ulcerated control group

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

ChrIII-100: Chromium III at 100ppm

ChrVI-10: Chromium VI at 10ppm

ChrVI-100: Chromium VI at 100ppm

4.13 EFFECT OF CHROMIUM ON SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN INDOMETHACIN INDUCED ULCER MODEL

The Superoxide Dismutase (SOD) activity of the nCont: 124.46±0.59; ChrIII-10: 115.69±1.10; ChrIII-100: 111.46±0.77; ChrVI-10: 123.15±0.09 and ChrVI-100: 121.46±0.27 were significantly higher than the ulcerated control (uCont) (108.58±0.49) group (Figure 15)

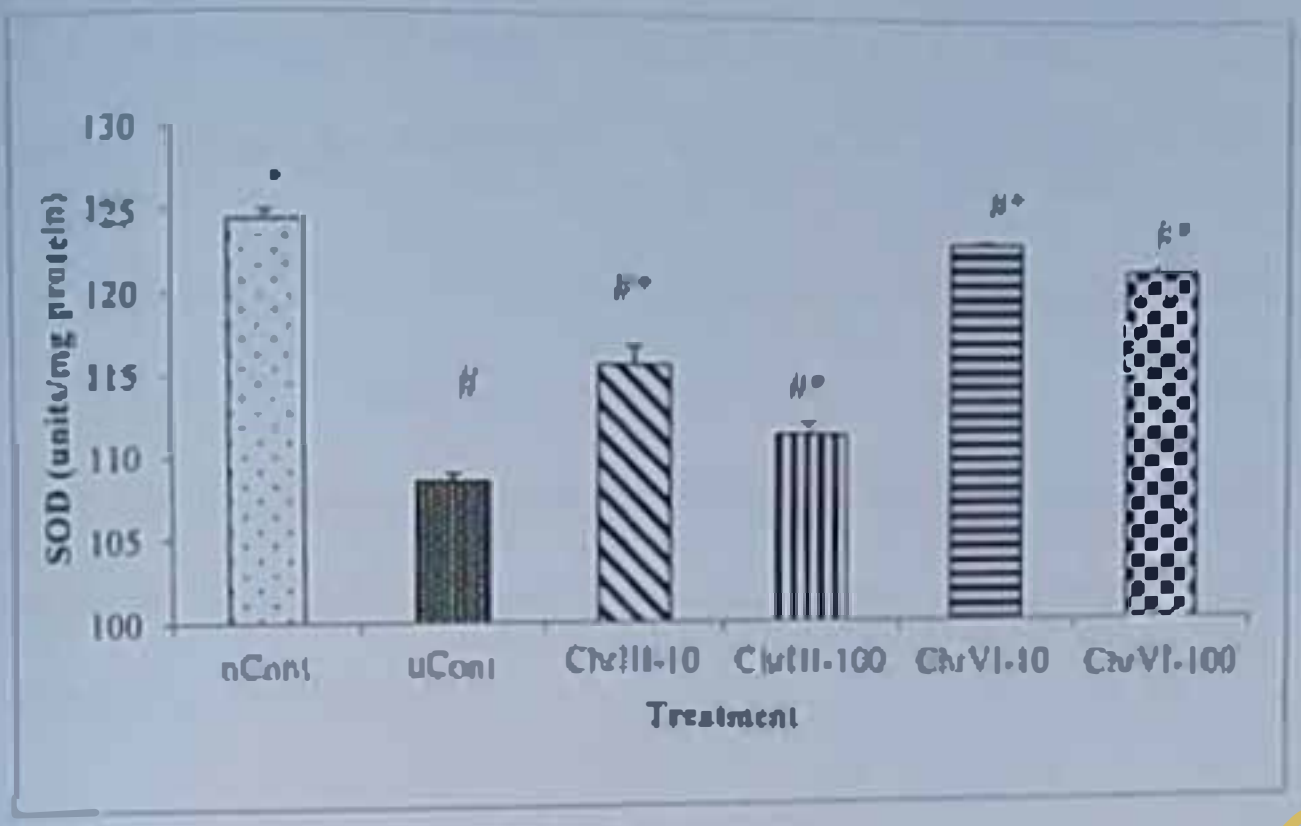


Figure 15. Effect of Chromium on Superoxide dismutase (SOD) activity in indomethacin induced ulcer model

* Significant at p<0.05 when compared with the Ulcerated control group

Significant at p<0.05 when compared with the non-ulcerated control group

nCont: Non-ulcerated control

uCont: Ulcerated control

CrIII-10: Chromium III at 10ppm

CrIII-100: Chromium III at 100ppm

CrVI-10: Chromium VI at 10ppm

CrVI-100: Chromium VI at 100ppm

4.14 EFFECT OF CHROMIUM ON CATALASE ACTIVITY IN INDOMETHACIN INDUCED ULCER MODEL

The Catalase activity was significantly higher in all chromium exposed groups (ChrIII-10: 1160.56±21.87; ChrIII-100: 1126.31±5.06; ChrVI-10: 1254.04±5.22; ChrVI-100: 1246.92±11.71) compared with the ulcerated control (uCont) (1094.31±8.28). They were however significantly lower compared to the non-ulcerated control (nCont) group (1497.52±14.02) (Figure 16).

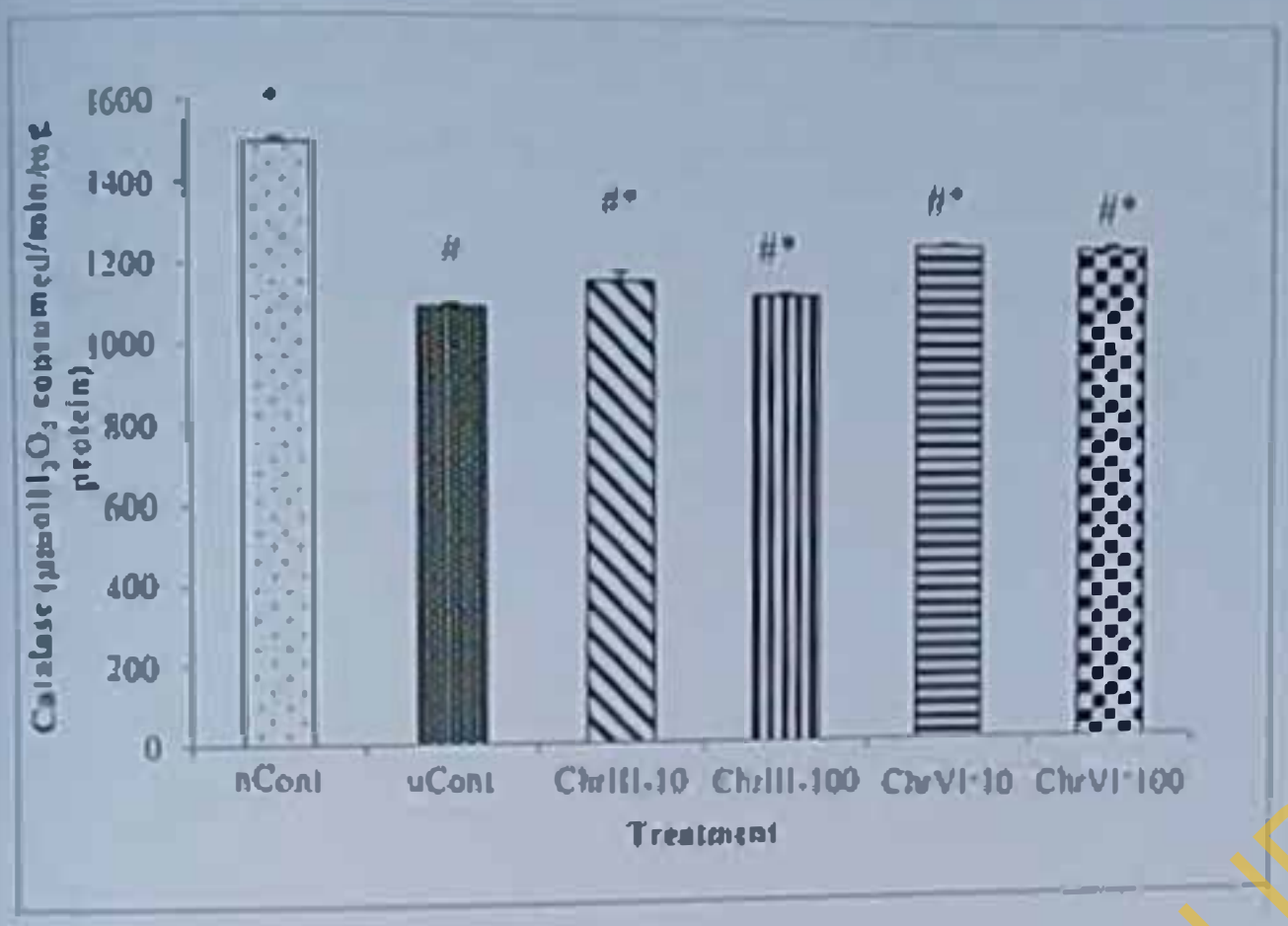


Figure 16: Effect of Chromium on catalase activity in indomethacin induced ulcer model

nCont: Non-ulcerated control

uCont: Ulcerated control

ChrIII-10: Chromium III at 10ppm

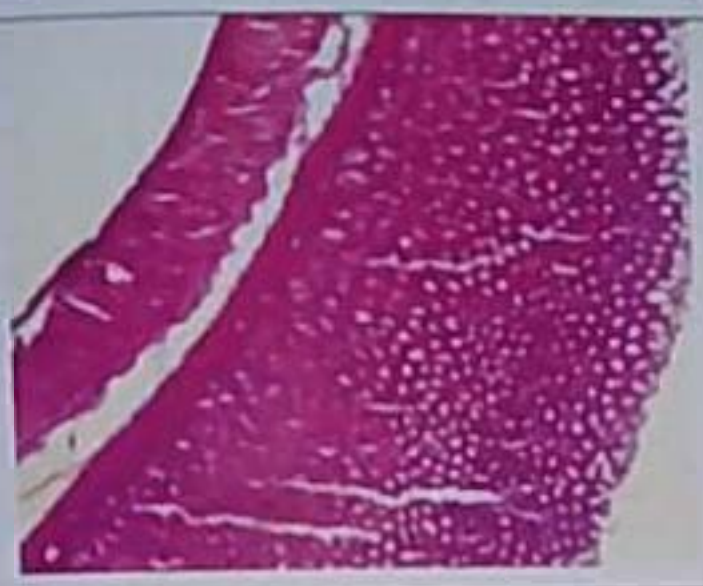
ChrIII-100: Chromium III at 100ppm

ChrVI-10: Chromium VI at 10ppm

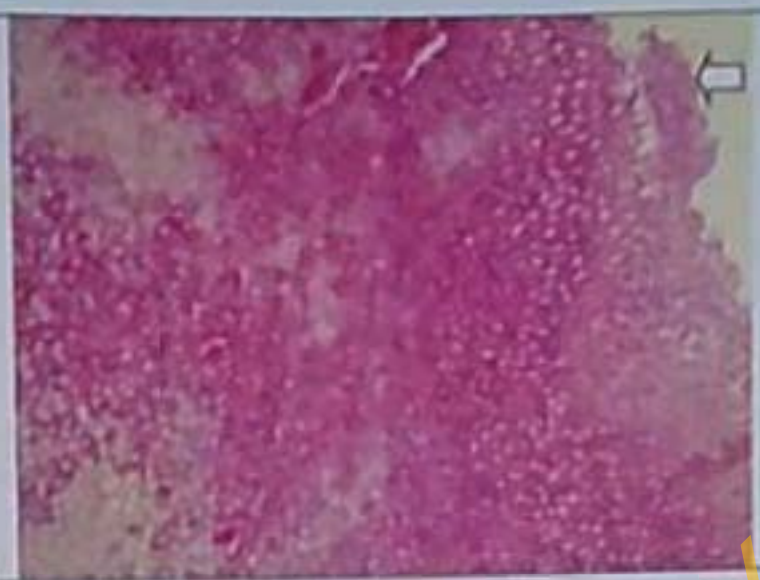
ChrVI-100: Chromium VI at 100ppm

4.15 PHOTOMICROGRAPH OF STOMACH HISTOLOGY IN INDOMETHACIN INDUCED ULCER MODEL

Histology showed erosion of the surface epithelium and marked congestion of blood vessels in the uCont group. The chromium exposed groups showed mild erosion of the surface epithelium as shown in Figure 17.



nCont: There is no visible lesion and the tunics are intact. H&E x100

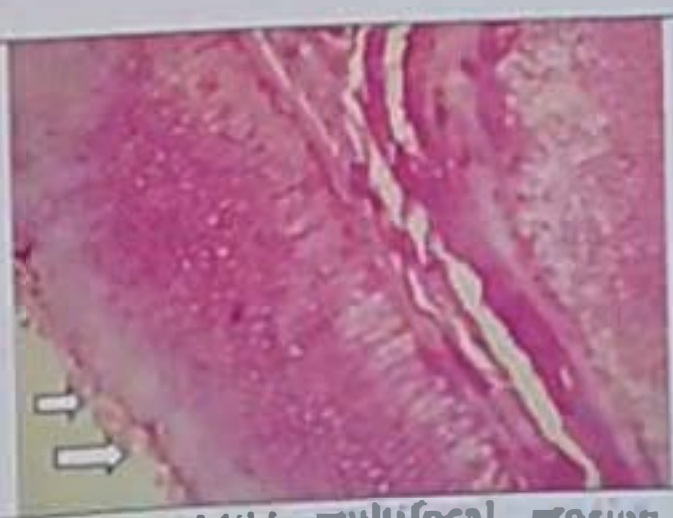


uCont: Erosion of surface epithelium (White arrow). H&E X100

ChrIII-10: A few foci of deep healed or depressed erosions; moderate congestion of blood vessels in submucosa (White arrow). H&E X100

ChrIII-100: A few foci of haemorrhages, necrotic foci being replaced by regenerating crypts (White arrow). H&E X100

ChrVI-10: Moderate erosion of surface epithelium (White arrow). H&E X100



ChrVI-100: Mild multifocal erosion of surface epithelium (White arrow). H&E X100

Figure 17: Photomicrographs of stomach tissues in indomethacin induced ulcer model

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study investigated the effects of Chromium on experimental ulceration using pyloric ligation model and Indomethacin induced model.

The administration of Chromium for the duration of twelve weeks caused a significant increase in the blood Chromium concentration in the Chromium exposed groups when compared with the control group. The increased blood Chromium level indicates exposure to Chromium and this increment observed is consistent with previous studies that observed elevated levels of Chromium in the blood, serum, urine, and other tissues and organs in patients with cobalt-chromium knee and hip arthroplasts (Coleman *et al.*, 1973; Michel, *et al.*, 1987; Sunderman *et al.*, 1989; ATSDR, 2000).

However, the increased blood Chromium concentration in the 100ppm Chromium VI group was significantly higher than the other Chromium exposed groups. This is in agreement with previous studies in both animals and humans that showed that exposure to hexavalent Chromium (Chromium VI) via drinking water leads to elevated Chromium levels in tissues, particularly the gastrointestinal tract, blood, liver, kidneys and spleen (Kerger *et al.*, 1996; Finley, 1997; Anderson, 2002; NTP, 2008; EPA, 2010a). Generally, Chromium VI compounds are better absorbed through the intestinal mucosa than the Chromium III compounds. In humans and animals, less than 1% of inorganic Chromium III and about 10% of inorganic Chromium VI are absorbed from the gut; the latter amount is slightly higher in a fasting state (Donaldson and Barreras, 1966; Dayan and Paine, 2001; ATSDR, 2008).

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due in an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors (Hoogerwerf nnd Pasricha, 2006; Sakat *et al*., 2012).

Pylorus ligature is an important procedure that shows the possible changes in parameters relative to the gastric content (Muriel *et al*., 2008; Sakat *et al*., 2012). The causes of gastric ulcer in pyloric ligation are believed to be due to increase in gastric hydrochloric acid secretion and/or stasis of acid this leading to auto digesuon of the gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforauon and haemorrhage (Kumar *et al*., 2011; Sakat *et al*., 2012).

In this study, the ligation of the pylorus end of the stomach causes obvious gastric macroscopic nnd microscopic mucosal injury. However, the exposure of the rats to Chromium III and Chromium VI at both 10ppm and 100ppm for the penod of twelve weeks significantly decreased gastric acid output and ocidity of the gastric juice compared to ulcerated control. This also significantly reduced the number of ulcers formed compared to ulcerated control. This significant decrease in the macroscopic appearance of the stomach in all Chromium exposed groups suggests that Chromium inhibits the formation of ulcer. Hexavalent Chromium seems to be protecong the stomach from mucosal injury caused by the accumulation of gastric juice in the stomach. This is contrary to the result of Mancuso (1951) in the study conducted on 97 workers in a chrome plant, he reported that gastrointestinal radiography revealed 10 of the workers had ulcer formation.

The chromium VI compounds are more readily absorbed, Chromium III is poorly absorbed by any route and the toxicity is attributed to Chromium VI form (ATSDR, 2008). The significant decrease in macroscopic ulcer score might be due to the reduction of Chromium

VI to Chromium III within the gastric environment where gastric fluid (De Flora *et al.*, 1987) and ascorbate (Samitz, 1970) play important roles (ATSDR, 2000). The first defense against Chromium VI after oral exposure is the reduction of Chromium VI to Chromium III within the gastric environment (ATSDR, 2000; ATSDR, 2008).

Gastric acid secretion is well known to play a role in gastric ulcer formation and this explains the mechanism of action of many anti-ulcer drugs (Schmassmann, 1998; Ajeigbe *et al.*, 2008). This confirms the significant increase in the gastric acid output in the ulcerated control group. However, the gastric acid output in all the groups were significantly decreased when compared with the ulcerated control group with the exception of the 100ppm Chromium VI which was lower to the ulcer alone group but was not significant. The decrease in the gastric acid output in the chromium exposed groups also confirms the reduction in the macroscopic ulcer score gotten from this study.

These results imply that exposure of rats to Chromium salts at doses of 10ppm and 100ppm prevents the formation of ulcer in the rat's stomach.

Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin cause gastric ulceration by inhibiting the synthesis of gastric prostaglandin (Goodman and Gilman, 1996; Wallace, 2001; Kleinman, 2008; Shirisha and Subash, 2012). These NSAIDs cause gastric ulceration by inhibiting the enzymes cyclooxygenase that promote synthesis of prostaglandins (Kushima, 2009; Sakat *et al.*, 2012). Prostaglandins are a group of physiologically active lipid compounds that have diverse hormone-like effects in animals and humans. Prostaglandins play a vital protective role in the stomach, by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair (Hayllar, 1995; Sakat *et al.*, 2012). Prostaglandin stimulates the development of a protective gel-like layer on the surface of the stomach. This gel layer

(mucin layer) protects stomach from stomach acid. The suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastric mucosal lesions (Maffinetheimer *et al*., 2000; Sakat *et al*., 2012). Indomethacin also causes ulcer by impairing the mucosal healing and decreasing the replication of stomach cells. Indomethacin also causes damage by the topical irritant effects of the drug on the epithelium (Wallace, 2000). It may cause direct irritation to the surface of the stomach.

In experimental rats, indomethacin causes the formation of gastric ulcer lesions. This is in agreement with the report of several authors on the role of indomethacin on gastric ulcer and erosion formation (Reeves and Stubbs 1985; Christopher *et al*., 1998; Ajayi *et al*., 2008).

The exposure of the experimental rats to Chromium III (10ppm and 100ppm) in indomethacin induced model led to a significant decrease in the mean macroscopic ulcer score of the rats compared to ulcerated control. However, the decrease in the mean ulcer score observed in the Chromium VI (100ppm and 10ppm) exposed groups was not significantly different from the ulcerated control group. The decrease in the mean ulcer score can also be explained based on the reduction of Chromium VI to Chromium III within the gastric environment.

Ulcer score results were further buttressed by histological evaluation which revealed mild erosion of surface epithelium in the chromium treated groups against observed visible lesions in ulcerated control group.

Lipid peroxidation, as measured by the amount of thiobarbituric acid reactive substances (TBARS) formed. Lipid peroxidation has been shown to be implicated in the aetiology of damage to subcellular membranes and then injury in the cell (Okaleye *et al*., 2007). Malondialdehyde (MDA) serves as an indicator to assess to assess oxidative damage of cells and tissues. In both ulcer models, lipid peroxidation, was decreased by Chromium exposure

in both ulcer models compared to the ulcerated alone group. This decrease in lipid peroxidation implies that Chromium causes a decrease in the formation of free radicals. The increase in the MDA level of the ulcerated control suggests the high free radical activity in the group and a decline in the enzymatic activity of superoxide dismutase and catalase.

The effect of chromium III and VI on experimental ulceration inhibits lipid peroxidation. The inhibition of enzymatic lipid oxidation may be achieved by inhibition of either the activation or reaction of an enzyme.

Antioxidants are scavengers which mop up free radicals that predispose tissues to inflammation (Olaleye and Ajeigbe, 2009). Antioxidants help to protect cells from damage caused by oxidative stress and enhance the body's defence systems against degenerative diseases (Bardi *et al.*, 2011). Superoxide dismutase (SOD) acts as the first line of defence against deleterious effects of oxyradicals that damage membrane structures while catalase converts the hydrogen peroxide generated to water, and molecular oxygen in order to protect membranes from lipid peroxidation. The superoxide dismutase (SOD) and catalase activities of Chromium exposed rats were significantly increased in this study which suggests the attenuating tendencies of chromium on ulcer formation.

In this study hexavalent chromium (Chromium VI) reduces the formation of gastric ulcer which might be due to poor absorption of chromium compounds within the gut. The absorption of trivalent chromium is less than 1% and that of hexavalent chromium is about 10% (Dernaldson and Barreras, 1966; Dayan and Paine, 2001; ATSDR, 2008). The reduction in ulcer formation can also be due to reduction of Chromium VI to Chromium III within the gastric environment.

5.2 CONCLUSION

From this study, it can be concluded that chromium attenuates the effect of pyloric ligation and indomethacin induced ulceration in rats, by decreasing the mean ulcer score, gastric output acidity and lipid peroxidation and also by increasing activities of catalase and superoxide dismutase. Chromium offers certain degree of protection against gastric injury induced by pylorus ligation and indomethacin via reduction of gastric oxidative stress.

5.3 CONTRIBUTION TO KNOWLEDGE

The oral exposure to Chromium III and Chromium VI at 10ppm and 100ppm protects the stomach by inhibiting the formation of reactive oxygen species.

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APPENDIX

Table 5: Blood Chromium concentration at 12 weeks

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean±SEM	0.08±0.01	0.08±0.01	0.12±0.01*	0.12±0.01*	0.13±0.01*	0.22±0.03*

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Table 6: Effect of Chromium on mean ulcer score in pyloric ligation ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean±SEM	0.00±0.00	4.00±0.45	1.63±0.24*	2.75±0.14*	1.75±0.25*	2.75±0.25*

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Table 7: Effect of Chromium on Gastric acid volume in pyloric ligation ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean± SEM	0.00±0.00	1.33±0.22	0.55±0.04*	0.80±0.04*	0.45±0.07*	1.05±0.03

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Table 8: Effect of Chromium on Gastric output acidity in pyloric ligation ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean±SEM	0.00±0.00	34.00±1.00	12.00±1.00*	26.92±2.47*	16.00±1.53*	26.75±1.46*

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Table 9: Effect of Chromium on lipid peroxidation in pyloric ligation ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean±SEM	3.24±0.13*	11.87±0.07#	8.19±0.08*#	8.95±0.28*#	8.90±0.29*#	9.72±0.17*#

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Table 10: Effect of Chromium on Superoxide Dismutase (SOD) activity in pyloric ligation ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean±SEM	124.58±1.01*	92.15±0.80#	115.34±1.35*#	99.71±2.10*#	106.72±0.88*#	95.02±0.16*#

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Significant at p=0.05 when compared with the non-ulcerated control (nCont)

Table 11: Effect of chromium on catalase activity in pyloric ligation ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean± SEM	1508.01± 20.22*	930.74± 9.28#	1173.95± 10.92*#	1021.25± 13.68*#	1089.10± 7.20*#	974.42± 6.82*#

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Significant at p=0.05 when compared with the non-ulcerated control (nCont)

Table 13: Effect of Chromium on Mean ulcer score in Indomethacin induced ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean±SEM	0.00±0.00	12.60±0.48	10.25±0.48*	10.78±0.18*	11.38±0.47	12.10±0.56

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

Table 14: Effect of Chromium on Lipid peroxidation in indomethacin ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean±SEM	3.21±0.09*	6.34±0.06#	4.88±003*#	5.29±0.11*#	5.58±0.04*#	5.96±0.03*#

* Significant at p=0.05 when compared with the Ulcerated control (uCont)

#Significant at p=0.05 when compared with the non-ulcerated control (nCont)

Table 15: Effect of Chromium on Superoxide dismutase (SOD) activity in indomethacin induced ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean± SEM	124.46± 0.59*	108.58± 0.49#	115.69± 1.10*#	111.46± 0.77*#	123.15± 0.09*#	121.46± 0.27*#

* Significant at p<0.05 when compared with the Ulcerated control group

Significant at p<0.05 when compared with the non-ulcerated control group

Table 16: Effect of Chromium on catalase activity in indomethacin induced ulcer model

GROUP	nCont	uCont	ChrIII-10	ChrIII-100	ChrVI-10	ChrVI-100
Mean± SEM	1497.52± 14.02*	1094.31± 8.28#	1160.56± 21.87*#	1126.31± 5.97*#	1254.04± 5.22*#	1246.92± 11.71*#

* Significant at p<0.05 when compared with the Ulcerated control (uCont)

Significant at p<0.05 when compared with the non-ulcerated control group (nCont)