GASTROPROTECTIVE EFFECTS OF ZINC AND VITAMIN E ON SOME BIOCHEMICAL AND HISTOLOGICAL CHANGES ON ETHANOL-INDUCED MUCOSAL DAMAGE IN WISTAR RATS

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Declaration

I hereby declare that the work in this thesis titled "Gastroprotective Effects of Zinc and Vitamin E on Some Biochemical and Histological Changes on Ethanol-induced Mucosal Damage in Wistar Rats" was performed by me in the Department of Biochemistry, under the Supervision of Prof. I. A. Umar and Mr. O. A. Owolabi. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

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This thesis titled "GASTROPROTECTIVE EFFECTS OF ZINC AND VITAMIN E ON SOME BIOCHEMICAL AND HISTOLOGICAL CHANGES ON ETHANOL-INDUCED MUCOSAL DAMAGE IN WISTAR RATS" by Oladunni, OLAJIDE meets the regulations governing the award of the degree of Master of Science degree of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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Abstract

Antiulcerogenic effects of zinc, vitamin E and their combination on ethanol-induced ulcerogenic rats were investigated using standard methods. The rats treated with the combination of zinc and vitamin E, followed by those treated with zinc alone and those treated with the standard drug, cimetidine had significantly ($P < 0.05$) higher anti-ulcerogenic properties as indicated by their preventive indices of 92.44 %, 89.92 %, and 73.11 % respectively compared to the rats treated with vitamin E alone (49.58 %). Antioxidant studies revealed that vitamin E (10.01 ± 0.54 µmol/g tissue), zinc (8.77 ± 0.55µmol/g tissue) and their combination (8.52 ± 0.87 µmol/g tissue) exerted higher protective effects against lipid peroxidation, which was significantly ($P < 0.05$) lower than the ulcerogenic rats that were not treated and which had a value of 11.30 ±0.15 µmol/g tissue. Superoxide dismutase (2.08 ± 0.10 U/g tissue) and glutathione peroxidase (43.17 ± 0.38 U/g tissue) activities increased significantly ($P < 0.05$) in the ulcerogenic groups treated with vitamin E, compared with the normal control group (1.63 ± 0.14 U/g tissue; 39.61 ± 3.18 U/g tissue). Catalase activity in the group treated with vitamin E (49.61 ± 1.73 U/g tissue) showed no significant ($P > 0.05$) difference compared with the normal control group (48.55 ± 2.76 U/g tissue) but was significantly ($P < 0.05$) different from the group treated with zinc (42.64 ± 2.73 U/g tissue). There was a significant ($P < 0.05$) increase in the packed cell volume (56.2 ± 3.01 %), haemoglobin concentration ((17.62 ± 0.76 g/dl) and red blood cell count (10.22 ± 0.71 x 10^6/µl) of the ulcerogenic rats without treatment compared to all the other treatment groups and the normal control group.The mean corpuscular volume of the treatment groups indicated a significant ($P < 0.05$) increase (Vitamin E - 61.63 ± 0.22 fl; Zinc alone - 60.97 ± 0.73 fl; and Zinc+ Vitamin E - 60.63 ± 1.53 fl.) as compared to the normal control (55.07 ± 0.64 fl) and ulcerogenic rats without treatment (57.20 ± 0.96 fl). The leucocyte count decreased significantly ($P < 0.05$) in the treatment (Zinc - 7.2 ± 0.79 x 10^3/µl; Vitamin E - 5.8 ± 1.2 x 10^3/µl) and normal control groups (6.1 ± 1.3 x 10^3/µl) as compared to the ulcerogenic rats without any treatment (17.7 ± 1.7 x 10^3/µl). The result of the study showed that zinc and/or vitamin E has antiulcerogenic properties and that zinc and vitamin E enhance the integrity of blood cells in ulcerogenic rats. The results showed that vitamin E and zinc supplements are of great value in the development of new anti-ulcerogenic agents once the appropriate dosage and ratio are utilized.

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ABBREVIATIONS

ANOVA: Analysis of Variance

BHT: Butylated Hydroxy-toluene

CAT: Catalase

COX: Cyclooxygenase

DNA: Deoxyribonucleic acid

ECL: Enterochromaffin-like cells

EDTA: Ethylenediaminetetra-acetic acid

ET.1: Endothelin-1

GPx: Glutathione Peroxidase

GSH: Reduced Glutathione

GSSH: Oxidized Glutathione

Hb: Haemoglobin Concentration

HCl: Hydrochloric acid

MCV: Mean Corpuscular Volume

MDA: Malondialdehyde

MUI: Mean Ulcer Index

NDGA: Nordihydroguaretic Acid

NO: Nitric Oxide

NSAIDs: Non-steroidal Anti-inflammatory Drugs

OD: Optical Density

PBS: Phosphate-buffered saline

PCV: Packed Cell Volume

PGs: Prostaglandins

PI: Preventive Index

PPI: Proton-pump Inhibitor

RBC: Red Blood Cell

RNA: Ribonucleic acid

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

SEM: Standard Error of Mean

SOD: Superoxide dismutase

TA: Total Area

TBARS: Thiobarbituric Acid-Reactive Substances

TCA: Trichloroacetic acid

UA: Ulcerated Area

UI: Ulcer Index

WBC: White Blood Cell

CHAPTER ONE

1.0 INTRODUCTION

Zinc and vitamin E are important components of biological antioxidant systems (Sahin and Kucuk, 2003). Zinc is required for growth, optimum performance and modulation of immune system, partly due to its role as a co-factor of more than two hundred enzymes (Zago and Oteiza, 2001). An antioxidant is any agent that limits the deleterious effects of free-radical stimulated oxidant reactions. An example is vitamin E which terminates free-radical-initiated chain reactions by donating electrons to radicals (Burton and Traber, 1989). Zinc, however, limits oxidant-induced damage in other ways through stabilization of membranes, which increases membrane resistance to oxidant damage and may down-regulate radical production. (DiSilvestro, 2000). The mechanism by which zinc exerts its antioxidant action is not well defined, but it has been suggested that it increases the synthesis of metallothionein, a cystein-rich protein, which acts as a free-radical scavenger` (Webb and Cain, 1982; Oteiza *et al.*, 1996). Kim *et al.* (1998) proposed another mode of action for zinc as an antioxidant by studying its interaction with vitamin E because; vitamin E status is impaired in zinc-deficient animals (Kim *et al.*, 1998). Zinc is capable of occupying iron and copper-binding sites on lipids, proteins and DNA; and, thus, exerts a direct antioxidant action (Tate *et al.*, 1999). Zinc supplementation decreases malondialdehyde (MDA) concentrations (Anderson *et al.*, 2001; Sahin and Kucuk, 2003).

Zinc has been recognized as a micronutrient of outstanding and diverse biological, clinical and global public health importance (Hambidge *et al.*, 2010). The regulation of absorption

by zinc transporters in the enterocytes is the principal means by which zinc homeostasis is maintained in the whole body (Hambidge *et al*., 2010).

Vitamin E is a major lipid soluble antioxidant present in all cellular membranes which protect against lipid peroxidation (Machlin, 1980; Ambali *et al.,* 2010b). Vitamin E functions as a chain-breaking antioxidant by preventing chain initiation and propagation of free-radical reactions and lipid peroxidation in cell membranes (Kamal-Eldin and Appelqvist, 1996). Vitamin E, therefore, plays an important role in cell survival by scavenging free radicals and other reactive substances (Halliwell, 1994). It has been suggested as a universal stabilizer of biological membranes in normal oxygen metabolism and peroxidation, as well as in metabolic disorders. Thus, vitamin E plays a vital role in the stabilisation of cell membranes and maintainance of their permeability (Navarro *et al.,* 1999; Ambali, *et al.,* 2010b). A decline in vitamin E in the serum leads to an increased oxidative status and enhanced susceptibility (Amazan *et al.,* 2012).

Gastro-intestinal mucosal cells rely on both luminal and bloodstream sources of nutrients for their nutrition. “Functional foods” is a term used to describe nutrients that have an effect on physiologic processes that is separate from their established nutritional function. Some of these nutrients are proposed to promote gastro-intestinal mucosal integrity; and they include amino acids, glutamine and arginine; essential micronutrients vitamin A and zinc; and two classes of food additives, prebiotics and probiotics (Duggan *et al*., 2002). Several types of glands provide different types of secretions in the alimentary tract, most of which act as lubricants to protect the stomach mucosa from excoriation. The pathophysiology of

gastric ulcer disease is often described as an imbalance between aggressive factors and mucosal protective mechanisms (Magaji *et al*., 2008). One of the characteristic features of the gastric mucosa in common with other epithelial tissues of the gastro-intestinal tract is a continuous layer of mucus covering its entire surface (Magaji *et al*., 2008). *Helicobacter pylori,* cigarette smoking, alcohol use and non-steroidal anti-inflammatory drugs (NSAIDs) are factors that impair mucosal protective layers (Huang *et al*., 2002). Gastric injury induced by ethanol is associated with significant production of oxygen free radicals which leads to increased lipid peroxidation, thus causing damage to cell and its membrane (Vinothapooshan and Sundar, 2010). Acute alcohol consumption at concentrations of over twenty-five percent has been shown to alter the gastric mucosal barrier and also to stimulate acid secretion; thus, alcohol may sensitize the mucosa to induce damage. Alcohol damages the epithelium by alterations in epithelial transport, intercellular junction disorders, and impairment of the mucosal barrier; all of which, in turn, facilitate hydrogen ion penetration into the mucosa (Bujanda, 2000). There is increasing evidence, indicating that chronic alcoholism is associated with increased oxidative stress and free radical-associated injury (Ligha and Fawehinmi, 2009).

1.1 STATEMENT OF RESEARCH PROBLEM

Peptic ulcer is a disease which is common worldwide (Pavo *et al*., 2000). Most patients plagued with this disease always have an incidence of recurrence even after treatment (Okasha *et al*., 2012). Although there are several drugs used for its treatment, they have several adverse reactions (Okasha *et al*., 2012), therefore, there is increasing interest in the

use of micronutrients such as zinc in the treatment of peptic ulcer due to its antioxidant role (Duggan *et al*., 2002).

1.2 JUSTIFICATION

Data are available on the use of different plants and some antioxidants in the treatment of ulcer, but there is no documented work on the use of zinc in combination with other known antioxidants and their effects on the gastro-intestinal lining. There is the need to compare the effects of these antioxidants to determine their possible synergestic effect. This research may shed more light on the role of zinc and vitamin E in the amelioration of ulcer.

The significance of the findings of the study will be as follows:

i) The results may shed more light to the current understanding of the pathogenesis and the role of oxidative stress in ulcer.
ii) The results of this study may be of value in the development of strategies, aimed at ameliorating the adverse effects of ulcer induced in the gastric mucosa.
iii) It may be of value in the determination of optimum time of therapeutic intervention against oxidative stress in Wistar rats, which may be of value in the management of the disease in other mammals and higher animals, including man.
iv) On the overall, the findings may improve the current approach to the diagnosis, prevention and treatment of ulcer.

1.3 AIM AND OBJECTIVES

The general aim is to determine the protective effect of zinc and vitamin E on ethanol-induced ulcer in Wistar rats.

The objectives are to:

i) Evaluate the effect of zinc and vitamin E on ethanol-induced gastric mucosal damage.

ii) Examine the effects of zinc and vitamin E as antioxidants in relieving oxidative stress in ulcerated rats.

iii) Determine the effects of zinc, vitamin E and the combination of both on the haematological parameters of the rats.

1.4 HYPOTHESIS

Zinc and vitamin E do not have significant effect in the amelioration of ethanol-induced ulcer.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTOLOGY AND ANATOMY OF THE STOMACH

The stomach in the monogastric animal is a thick-walled organ that lies between the oesophagus and the first part of the small intestine (the duodenum). It is on the left side of the abdominal cavity; the fundus of the stomach lies against the diaphragm. Lying beneath the stomach is the pancreas. The stomach is divided into four sections, each of which has different cells and functions. The sections are: Cardiac region, where the contents of the oesophagus empty into the stomach. The fundus, formed by the upper curvature of the organ; the body, the main central region, and pylorus or atrium (Ernst, 1997). The stomach comprises of four layers which are also known as tunics. Each layer has different tissues and functions. From the inside to the exterior the tunics are called: mucosa, submucosa, muscularis, and serosa. The mucosa consists of an epithelium, the *lamina propria* underneath, and a thin bit of smooth muscle called the *muscularis mucosa*. The submucosa lies under the *muscularis mucosa* and consists of fibrous connective tissue, which separates the mucosa from the next layer, the *muscularis externa.* The muscularis in the stomach differs from that of other gastrointestinal organs because it has three layers of muscle instead of two. Under these muscle layers is the adventitia, layers of connective tissue, continuous with the omenta. The epithelium of the stomach forms deep pits, called fundic or oxyntic glands. Different types of cells are at different locations down the pits. The cells at the base of these pits are chief cells, responsible for production of pepsinogen, an inactive

precursor of pepsin, which degrades proteins. The secretion of pepsinogen prevents self-digestion of the stomach cells (Chen and Chen, 1989). Mucus secretion is regarded as a crucial defensive factor in the protection of gastric mucosa from gastric lesions (Oluwole *et al*., 2008), mucus serves as the first line of defence against ulcerogens. It is secreted by mucus neck cells and covers the entire gastrointestinal mucosa thereby preventing physical damage and back diffusion of hydrogen ions (Williams and Turnberg, 1980).

2.2 PHYSIOLOGY AND BIOCHEMISTRY OF THE STOMACH

Food has to be swallowed, processed and broken down (digestion) and taken up (absorption) by the intestine to cover the material and energy demands of living things (Silbernagl *et al*., 2000).The proximal stomach primarily serves to store food ingested, and its muscle tone determines the supply to the distal stomach, where the food is further broken down and emulsified (Silbernagl *et al.*, 2000). The stomach plays a major role in the digestion of food we eat (Fornai *et al*., 2011). The stomach can resist a large variety of noxious factors, including hydrochloric acid, refluxed bile salts and alcohol. The high resistance to injuries depends on a number of physiological responses elicited by the mucosal lining against potentially harmful agents, and the ability to repair rapidly the mucosal damage when it occurs (Laine *et al*., 2008). Proteins are denatured and broken down by the gastric acid and pepsin,while lipases begin fat digestion. The distal stomach also has the task of apportioning chyme. In addition, the stomach secretes the intrinsic factor that is essential for the absorption of cobalamines (vitamin B_{12}). (Silbernagl *et al*., 2000). The HCO_3^- ions of the pancreatic juice are needed to neutralize the acidic chyme. The products of digestion (monosaccharides, amino acids, dipeptides, monoglycerides and

free fatty acids), water, minerals and vitamins are absorbed in the small intestine (Silbernagl *et al*., 2000).

Acid secretion from the parietal cell represents a major function of the stomach. The secretion is controlled in a complex manner by at least three different gastric cells, the enterochromaffin-like (ECL) cells producing histamine, the G cell releasing gastrin, and the D cell releasing somatostatin (Tobin *et al.*, 2009). Furthermore, there exists a cholinergic vagal control, which is exerted on all the mentioned cells and directly on the parietal cells. Histamine, gastrin and vagally-released acetylcholine influence directly the production of acid from the parietal cells. Somatostatin inhibits gastrin and histamine release and, hence, acid production (Tobin *et al.*, 2009). The two plexuses in the wall of the oesophagus, stomach and intestine serve to control motility and secretion, with super-regional reflexes and modulating influences of the central nervous system, transmitted via the autononomic nervous system and visceral–afferent nerve tracts. In addition, the gastro-intestinal tract secretes numerous peptide hormones and neuro transmitters that participate in controlling and regulating the gastro-intestinal tract and its accessory glands (Silbernagl *et al*., 2000; Rumessen and Vanderwinden, 2003).

2.3 PATHOPHYSIOLOGY OF ULCER

The stomach is a site of massive production and concentration of reactive oxygen species (ROS) in a higher proportion, compared to other tissues or biological fluids (Graziani *et al*., 2005). The generation of these reactive oxygen species plays a major role in the development of multiple pathologies, such as gastritis, peptic ulcerations or gastric adenocarcinoma (Oliveria *et al*., 2003). Ulcer is the most prevalent chronic gastrointestinal

disorder in nature (Valle, 2008). Peptic ulcers are open sores which develop on the inner mucosal lining of the digestive tract; mainly, the initial portion of the small intestine, oesophagus and stomach (Dufton, 2012). Peptic ulcers occur with loss of mucus membrane of any part of the gastro-intestinal tract exposed to pepsin or acidic contents of the gastric juice (Umana *et al*., 2012). The pathophysiology of ulcer usually involves an imbalance between aggressive and defensive mucosal factors (Wallace and Sharkey, 2011). The aggressive factors include hydrochloric acid, pepsin and *Helicobacter pylori,* which are injurious (Lahiri and Palit, 2012). The defensive mucosal factors include: mucin, prostaglandin, bicarbonate, nitric oxide and growth factors (Lakshmi *et al*., 2009; Wallace and Sharkey, 2011). Gastric mucus consists of viscous, elastic adherent and transparent gel formed by water and glycoproteins. The protective effects of the mucous barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface (Iyyam Pillai *et al*., 2010). The ability of the gastric mucosa to resist injury caused by endogenous secretions (such as acid, pepsin and bile) and by ingested irritants such as alcohol, aspirin and NSAIDs can be attributed to factors which are generally called mucosal defence (Wallace, 2001) In healthy individuals, the digestive tract is coated with a mucous membrane that protects the underlying tissue against the higly corrosive digestive acid. If the amount of the acid is increased or the pH reduced significantly, the acid damages the tissue thus, leading to ulceration (Kurata *et al*., 1984).

2.4 PATHOGENESIS OF ULCER

The gastric epithelium is often attacked by physical, chemical or microbiological agents which act from the gastric lumen and these injurious agents and irritants are both exogenous

and endogenous in origin (Umana *et al*., 2012). Some of the factors involved in the pathology of ulcer include: stress, smoking, nutritional deficiencies, alcohol ingestion and non-steroidal anti-inflammatory drugs (Basil and Howard, 1995). The mechanisms of gastric mucosal defence include several local and neurohormonal protective factors, which allow the mucosa to resist against frequent exposures to damaging factors (Laine *et al.*, 2008). The mucosal defense mechanisms include: mucus-bicarbonate-phospholipid barrier, epithelial cells, mucosal cell renewal, mucosal blood flow, sensory innervations, prostaglandins and neurohormonal mechanisms (Fornai *et al*., 2011).

2.4.1 Agents involved in Ulcer Pathogenesis

There are many factors which play a major role in the pathogenesis of ulcerations. They include sedentary life style, alcohol intake, spicy food, drugs and several bacterial infections (Kumar *et al*., 2012). The gastric mucosa is continuously exposed to potentially injurious agents such as acids, pepsin, bile acids, food ingredients, bacterial products and drugs (Selvamathy *et al.,* 2010). Psycogenic factors such as stress play a major role in the pathogenesis of gastric ulcers in man (Lahiri and Palit, 2012). Ulcers are caused commonly by *Helicobacter pylori* and non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen (Ramakrishnan and Salinas, 2007). Other causes of ulcer include heavy consumption of alcohol, radiation therapy, burns and physical injury (Bhowmik *et al*., 2010).

2.4.1.1 *Helicobacter pylori*

Helicobacter pylori are bacterial organisms responsible for most ulcers (Bhowmik *et al*., 2010). It is a Gram-negative bacillus, motile, microaerophilic, flagellated and spiral shaped bacteria (Majumdar *et al*., 2011). *Helicobacter pylori* weaken the protective coating of the stomach and duodenum, thus allowing the damaging digestive juices to irritate the sensitive lining below (Graham *et al*., 1999).

2.4.1.2 *Non-steroidal anti-inflammatory drugs and Prostaglandins*

Non-steroidal anti-inflammatory drugs cause damage to the stomach mucosal lining by overcoming the expression of cyclooxygenase (COX) which has been shown to inhibit the conversion of amino acids to prostaglandins resulting in negative impact on mucosal defence (Lahiri and Palit, 2012). The inhibition therefore impairs the mucosal barrier with the end results in the progression of ulcers (Scarpignato and Hunt, 2010). In addition, COX-1 inhibition by non-steroidal anti-inflammatory drugs leads to significant release of the endothelin-1 (ET-1), which is a potent vasoconstrictor shown to induce mucosal injury (Whittle, 2002; Vane and Botting, 1998). The non-steroidal anti-inflammatory drugs, by inhibiting the prostaglandin synthesis, initiate gastric injury; prostaglandins cause the activation of neutrophils and the local release of ROS (Kumar *et al.*, 2012).

2.4.1.3 *Alcohol*

Alcohol is regularly consumed by majority of people living in advanced countries. The main reasons for consumption of alcohol are, to produce positive mood states and its stress relieving effects (Spanagel, 2009). Chronic consumption of alcohol has enormous health

and socio-economic impact on the world population (Niccols, 2007). Although most body organs are affected by alcohol intoxication and chronic alcohol use, severe alcohol-induced diseases are most notable in the liver, pancreas and brain (Spanagel, 2009). It has been demonstrated over the years that ethanol acts directly on the membrane receptors and ion channels (Jurd *et al.,* 2003). When alcohol is consumed, the alcoholic beverages first pass through the various segments of the gastro-intestinal tract and as such may interfere with the structure as well as the function of the gastro-intestinal tract segments (Bode and Bode, 1997). There is increasing evidence indicating that alcoholism is associated with increased oxidative stress and free-radical-associated injury (Manso, 1997). The break-down of alcohol in the liver results in the formation of molecules, whose further metabolism in the cells leads to increased generation of ROS (Toykuni, 1999). Alcohol stimulates the activity of enzymes called cytochrome P450s, which contribute to free-radical generation and also reduces the levels of agents, capable of eliminating ROS such as selenium, zinc and vitamin E (Ligha and Fawehinmi, 2009). Ethanol being a necrotizing agent, damages the superficial epithelial layers and inhibits the release of mucosal prostaglandins (Miller and Henagan, 1984; Lahiri and Palit, 2012). The mechanism of ethanol-induced gastric lesions is varied, including the depletion of gastric mucus content, damaged mucosal blood flow and mucosal cell injury (Kumar *et al*., 2012). Acute alcohol administration affects the histamine level in the brain, depending on the dose of alcohol, the species of experimental animal, and the brain structure (Zimatkin and Anichtchik, 1999). Alcohol consumption poses a risk for people who abuse alcohol, thus putting them in a danger of alcohol related complications, including those affecting blood cells and proteins, and the bone marrow (Ballard, 1989).

2.5 Mechanism of Action of Ethanol in the induction of Ulcer

Analyses in several animal species revealed that acute alcohol administration by mouth or by direct infusion into the stomach or blood may affect gastric acid secretion (Chari *et al.,* 1993). Alcoholic beverages with low alcohol content strongly increase gastric acid secretion and the release of gastrin, the gastric hormone that induces acid secretion (Bode and Bode, 1997). Alcohol causes about sixty different types of disease conditions (Anderson and Baumberg, 2006). Ethanol-induced acute gastric mucosal injury model suggests that ethanol, being a necrotizing agent, damages the superficial epithelial layers and inhibits the release of mucosal prostaglandins (Bode *et al*., 1996; Lahiri and Palit, 2012).

Prostaglandins are hormone-like substances which protect the gastric mucosa from damage (Bode and Bode, 1997). The mechanism of ethanol-induced gastric lesions varies and includes the depletion of gastric mucus content, damaged mucosal blood flow and mucosal cell injury (Kumar *et al.*, 2012). Ethanol causes severe damage to the gastro-intestinal mucosa; starting with microvascular injury, resulting in increased vascular permeability; to edema formation and epithelial lifting (Szabo *et al.,*1998). Several studies have demonstrated that alcohol increases lipid peroxidation as it has been reported to release superoxide anion and hydroperoxyl free radicals (Kumar *et al.,*2012), which lead to an increased lipid peroxidation (Mahmood *et al*., 2011). Increased lipid peroxide content and oxygen-derived-free radicals results in marked changes in cellular levels, and cause membrane damage, cell death, exfoliation and epithelial erosion (Umamaheswari *et al.,* 2007; Mahmood *et al*., 2011). Gastric lesions may be observed only after an hour of administration of alcohol (Lahiri and Palit, 2012). Severity of the mucosal involvement has

been assessed by counting the number of haemorrhagic spots or by scoring the mucosal lesions, which appear as blackish lesions, grouped in patches of varying sizes, usually parallel to the major axis of the stomach (Witt *et al.,* 1985; Stermer, 2002; Repetto and Llesuy, 2002).

2.6 PREVALENCE OF ULCER

Prevalence of ulcer is approximately 12% in men, and 10% in women; with about 15,000 deaths, occurring yearly as a result (Valle, 2008). In the treatment of ulcer today, there are two main approaches, which include: reduction of gastric acid secretion and reinforcement of gastric mucosal protection (Lahiri and Palit, 2012). Gastric ulcers are widespread in the various populations of many countries and several reports indicate that ROS or free-radical generation play an important role in the pathophysiological processes of acute gastric lesions (Vaananen, 1991; Ligha and Fawehinmi, 2009). There is an increasing body of evidence, which indicates that chronic alcoholism is associated with free-radical-mediated injury and, as such, it is associated with increased oxidative stress (Manso, 1997). Ethanol is a necrotizing agent, which damages the surface epithelial layers and inhibits the release of mucosal prostaglandins (Lahiri and Palit, 2012).

2.7 TREATMENT OF PEPTIC ULCER

The ultimate goal in the treatment of ulcer includes relieving pain, healing the ulcer and preventing complications; as such the first stage involves the reduction or eradication of the risk factors followed by medications (Marks, 2012). Ulcer medications include H_2- blockers or H_2-receptor antagonists, proton pump inhibitors (PPI), antibiotics and bismuth salts. The

method of treatment depends on the risk factor and the mechanism of action of the drug involved (Meurer and Bower, 2002). The discovery of the effective and potent suppressants and the identification of the largely bacteria cause of peptic ulceration are the two most important developments which are associated with the overall decrease in the rates of peptic ulcer disease (Malfertheiner *et al*., 2009).

2.7.1 H_2-blockers

H_2-blockers are also called histamine blockers or H_2-receptor antagonists. They are competitive antagonists of histamine at the parietal cell H_2-receptors in the stomach. H_2-blockers suppress the secretion of hydrochloric acid (HCl) by the parietal cells by two mechanisms: - Histamine released by enterochromaffin-like cells in the stomach, is blocked from binding on parietal cell H_2-receptors which stimulate acid secretion thus, other substances that promote acid secretion such as gastrin and acetylcholine have a reduced effect on the parietal cells when H_2-receptors are blocked (Dufton, 2012). Some H_2-blockers such as cimetidine interfere with the body's mechanism of drug metabolism and elimination through the liver cytochrome P450 pathway thus increasing the risk of drug toxicity and it has also been linked to hypotension, gynecomastia in males, loss of libido and impotence (Anon., 2009).

2.7.2 Proton-pump Inhibitors

These are a group of drugs which display long lasting reduction of stomach acid production. They are more potent than H_2-blockers (Marks, 2012). PPI act by irreversibly blocking the hydrogen/potassium adenosine triphosphatase enzyme system (gastric proton pump) of the

stomach's parietal cells. PPI reduce gastric acid secretions by up to 99 percent (Dufton, 2012). The lack of stomach acids aids in healing gastric ulcers and reduces pain from indigestion and heartburn, but HCl is required for the digestion of proteins and absorption of nutrients, especially vitamin B-12 and calcium, as such insufficient HCl (hypochlorydria) can lead to side effects such as B-12 deficiency, increased risk of bone fracture, increased risk of certain heart arrhythmias and interstitial nephritis, low serum magnesium levels, headaches, nausea, diarrhea, abdominal pain, flatulence, constipation, fatigue and dizziness, also there is a suggestion from more recent data of a rebound effect when PPI are discontinued. The common PPI include, omeprazole, lansoprazole, esomeprazole, pantoprazole and rabeprazole (Sachs *et al*., 2006).

2.7.3 Antibiotics

This class of drugs is prescribed to kill bacteria such as *Helicobacter pylori* but due to resistance and adaptability, more than one type is often recommended to be taken at the same time. Classes of antibiotics used include amoxicillin, metronidazole (flagyl), furazolidone (furoxone) and tetracycline. Antibiotics are usually prescribed for only two weeks at a time to avoid side effects. Some common side effects include acquired antibiotic resistance, serious allergic reactions, nausea, upset stomach, diarrhea, sun sensitivity, disruption of the intestinal flora and fauna, systemic overgrowth of pathogenic bacteria (such as *Clostridium difficile*) and yeast species (such as *Candida albicans*) (Dufton, 2012).

2.7.4 Bismuth Salts

This involves the use of medications that protect the lining of the stomach and small intestine from the damaging of acids. These agents are called protective agents and include sucralfate (carafate), misoprostol (cytotec) and Bismuth Salicylate (Pepto-Bismol). Bismuth Salicylate can cause severe bleeding problems when used alone in patients with bleeding ulcers (Anon., 2009).

2.8 OXIDATIVE STRESS, FREE RADICALS, REACTIVE OXYGEN AND NITROGEN SPECIES

2.8.1 Oxidative Stress

According to Halliwell and Gutteridge (2007), Oxidative stress refers to a state where there is an imbalance between the generation and neutralisation of ROS by antioxidants, caused by excessive production of ROS, loss of antioxidant defenses or both; it results from an increase in ROS, impairment of antioxidant defense system or insufficient capacity to repair oxidative damage (Aly *et al*., 2010). Oxidative damage plays a crucial role in the pathogenesis of various diseases like atherosclerosis, cancer, liver damage, rheumatoid arthritis, immunological incompetence, neurodegenerative disorders and ageing (Dalle-Donne *et al*., 2003; Salar-Amoli *et al*., 2009). Formation of ROS and reactive nitrogen species (RNS) that exceed the body's antioxidant capacity has been termed oxidative stress (Finaud *et al*., 2006). Under normal circumstances, cells are able to balance the production of oxidants and antioxidants, resulting in redox equilibrium (Korkmaz *et al.*, 2008). Oxidative stress occurs when cells are subjected to excess levels of ROS or as a result of depletion of antioxidant defences (Sies, 1997). It is a well known mechanism of cellular

injury that occurs with increased lipoperoxidation of cell phospholipids and has been implicated in various cell dysfunctions (Catala, 2006). Oxidative stress, resulting from increased production of free-radicals and ROS and/or decreased antioxidant defence, leads to damage of biological macromolecules, and disruption of normal metabolism and physiology (Trevisan *et al*., 2001; Nazifi *et al*., 2009). Many environmental and biochemical changes cause elevated ROS production, including hazardous contaminants such as nitroso compounds, polycyclic hydrocarbons, alcohol, aflatoxin and heterocyclic aromatic amines; tissue damage such as mechanical, heat and acid; infectious factors, inflammatory reactions, environmental hazards and biochemical changes generate excess ROS (Sies, 2007).

2.8.2 Free Radicals

Free radicals are molecules containing at least one unpaired electron in their outer orbit. They have a very important role in origin of life and biological evolution, leaving beneficial effects on the organisms (McCord, 2000). Free radicals arise by the haemolytic fission of a covalent bond where each fraction retains one electron through a process, which requires energy expense provided by high temperature or irradiation. The energetically less demanding oxidation (electron loss) or reduction (electron acceptation) constitutes the two main routes to free radicals (Pourova *et al.,* 2010). Free radicals consist of superoxide anion (O^{-}_{2}) and hydroxyl radical ($^{\cdot}OH$) and they together with H_2O_2 constitute ROS. Reactive nitrogen species (RNS) includes nitric oxide (NO) and peroxynitrite (Brambilla *et al.*, 2008). The physiological defence systems that counteract free-radicals encompass endogenous enzyme systems, such as catalase, glutathione reductase and superoxide

dismutase, and glutathione, urate and coenzyme Q, or exogenous factors such as β-carotene, vitamin C, vitamin E and selenium (Valko *et al*., 2006).

The most important biological free-radicals are; superoxide anion ($O^{-}_{2)}$, hydroxyl radical ($^{\cdot}OH$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$) and hydroperoxyl ($HO_2^{\cdot}$). Other ROS that are not free radicals include hydrogen peroxide (H_2O_2), ozone (O_3), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$) (Pourova *et al*., 2010). Overproduction of free radicals can cause oxidative damage to biomolecules (lipids, proteins, DNA), eventually leading to many chronic diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, post-ischaemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, ageing, gastro-intestinal inflammatory bowel disease, coeliac and and other degenerative diseases in humans (Fridovich, 1999; Stocker and Keany, 2004; Tsukahara, 2007; Castellani *et al*., 2008). Thus, free radicals are a family of highly reactive and diverse species, which are capable of extracting electrons. They therefore, oxidize a variety of biomolecules that are vital to tissue function. They include oxygen, nitrogen and chlorine species (Sharma and Sharma, 2011).

2.8.3 Reactive Oxygen and Nitrogen Species

Reactive oxygen species (ROS) is a collective term used for a group of oxidants, which are either free radicals or molecular species, capable of generating free radicals (Kunwar and Priyadarsini, 2011). ROS have extremely short half-lives, but have the ability to cause substantial tissue damage by initiating free radical chain reactions (Waddington *et al*., 2000). Under normal physiological conditions, almost two percent of the oxygen consumed

in the body is converted to superoxide (Wintebourn, 2008). Normally, a low concentration of ROS is essential for normal body functions such as gene expression, cellular growth and defence against infection (Dröge, 2002). ROS comprise hydrogen peroxide (H_2O_2), nitric oxide (NO), superoxide anions and the highly reactive hydroxyl and monoxide radicals (OH•, NO•) (Uttara *et al*., 2009). In all forms of ROS generation, molecular oxygen needs to be activated and cellular system have evolved range of metallo-enzymes that facilitate ROS generation upon interaction of redox metals with O_2 using various catalytic pathways. Since free radicals are toxic to cells, under normal circumstances, cells have efficient regulating system for O_2, and metal ion interaction, leading to free radicals and ROS generation (Bush, 2000). Superoxide anion, which is a product of a one-electron reduction of oxygen, is the precursor of most ROS and a mediator in oxidative chain reactions (Turrens, 2003). Oxygen may react with other radicals, including nitric oxide ($NO^{\cdot}$) in a reaction controlled by the rate of diffusion of both radicals, resulting in peroxynitrites which is a very strong oxidant (Radi *et al*., 2002); the oxidants derived from peroxynitrite are called RNS (Turrens, 2003). The NO radical is produced in higher organisms by the oxidation of one of the terminal guanodine nitrogen atoms of L-arginine (Dröge, 2002). Depending on the micro-environment, NO can be converted to various other RNS such as nitrosium cation (NO^+), nitroxyl anion (NO^-) or peroxynitrite ($ONOO^-$) (Franco and Estévez, 2012).

ROS are involved in many pathological processes, including cell mutations, cancer, some symptoms of ageing, diabetes, neuropathy, vascular problems, schizophrenia, Alzheimer's disease, alcohol-induced liver damage and cigarette-induced lung injury (Lamberth *et al*., 2007).

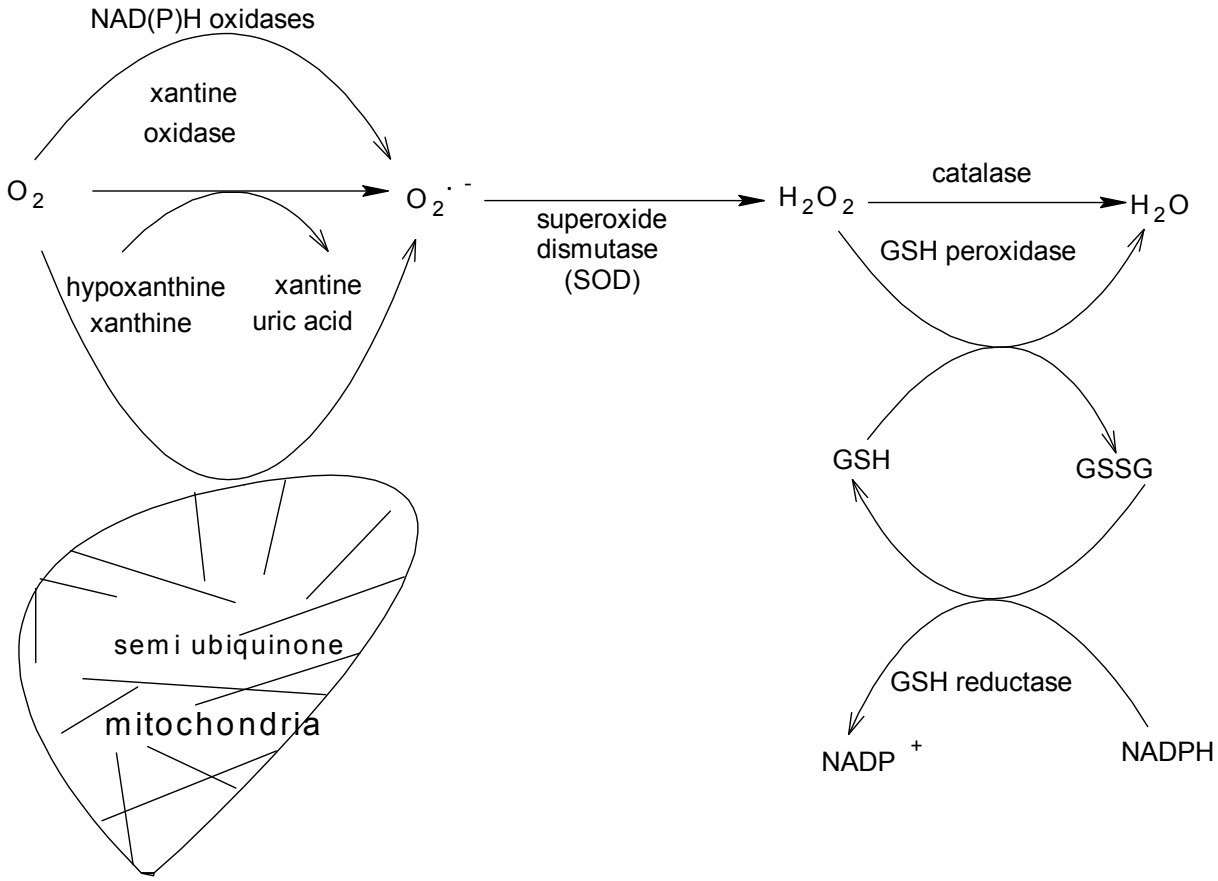


Figure 2.1. Pathways of Reactive Oxygen Species production and clearance (Dr□ge, 2002)

2.9 ANTIOXIDANTS

According to Halliwell and Gutteridge (2007), an antioxidant is a substance which when present at low concentration compared with that of an oxidizable substrate, inhibits or delays the oxidation of the substrate. Antioxidants are also classified as exogenous (natural or synthetic) or endogenous compounds, both responsible for removal of free radicals, scavenging ROS or their precursors, inhibiting formation of ROS and binding metal ions needed for catalysis of ROS generation (Uttara *et al.*, 2009). These substances include the enzymes, superoxide dismutase, glutathione peroxidase (GPx) and catalase; and non-enzymatic compounds such as α-tocopherol (vitamin E), β-carotene, ascorbate (vitamin C) and glutathione (Dröge, 2002). Antioxidants can be classified based on their function which are, free-radical scavengers (for example, ascorbic acid); scavengers of non-radical oxidants (for example, catalase or thiols); compounds that inhibit generation of oxidants (examples are, metal chelators); and compounds that induce the production of antioxidants (for example, isothiocyanates (sulforaphane)] (Winterbourn, 2009). There are also some compounds that have a relatively low specific antioxidative activity, but when present at high concentrations, may contribute significantly to the overall ROS scavenging activity. These compounds include: free amino acids, peptides and proteins (Siebenlist *et al*., 1994). Strong defence mechanisms in the form of enzymatic and non-enzymatic antioxidants against these free radicals must be developed in tissues to protect such damages effectively.

Endogenous enzymatic antioxidants like catalase, superoxide dismutase and glutathione peroxidase play a vital role in scavenging oxidative radicals and are therefore, considered as markers for the evaluation of oxidative stress (Maini *et al*., 2007). Non-enzymatic antioxidants include direct acting antioxidants, which are extremely important in defence against oxidative stress. Most of them include ascorbic and lipoic acid, polyphenols and

carotenoids, derived from dietary sources. The cell itself synthesizes a minority of these molecules. Indirectly acting antioxidants mostly include chelating agents and they bind to redox metals to prevent free radical generation (Sherki *et al*., 2001). Endogenous antioxidant defences are inadequate to completely minimize the on-going oxidative damage to DNA, lipids, proteins and other biological molecules. Hence, diet-derived antioxidants may be particularly important in protecting against diseases resulting from cellular damages (Anyasor *et al.*, 2009). Antioxidants may thus protect cells against the damaging effects of ROS, such as singlet oxygen, superoxide, peroxyl radicals and peroxynitrite (Asiedu-Gyekye *et al.*, 2012). Antioxidants safely interact with free radicals and terminate the chain reactions before vital molecules are damaged (Otitoju *et al*., 2008).

2.10 REACTIVE OXYGEN SPECIES AND TISSUE/CELL DAMAGE

Reactive oxygen species may cause damage to various cellular and extracellular tissues by targeting protein damage, lipid peroxidation, and DNA damage (Sharma and Sharma, 2011).

Protein damage results in fragmentation and polymerisation reactions of various protein molecules, which lead to the formation of protein radicals and protein-bound ROS (Weighardt *et al.*, 2000). Proteins can undergo direct and indirect damage following interaction with ROS, resulting into peroxidation, changes in their tertiary structure, proteolytic degradation, protein-protein cross linkages and fragmentation (Beckman and Ames, 1997). Lipid peroxidation which is one of the most important reactions of free radical species (Tsai *et al*, 2005) occurs as a result of the presence of high concentration of

unsaturated fatty acids in their lipid components (Kunwar *et al*., 2009). Lipid peroxidation is a non-enzymatic chain reaction based on the oxidation of mainly unsaturated fatty acids, and is associated with the presence of ROS (Erisir *et al*., 2009). ROS reactions with membrane lipids cause lipid peroxidation, resulting in the formation of lipid hydroperoxide (LOOH), which can further decompose to an aldehyde such as malonaldehyde, and 4-hydroxy nonenal (4-HNE) (Repetto *et al*., 2012) or form cyclic endoperoxide, isoprotans and hydrocarbons, with the resulting consequences of cross linking of membrane proteins, change in membrane fluidity, formation of lipid-protein, and lipid-DNA adduct, which may be detrimental to the functioning of the cell (Catala, 2006). The intermediates formed may influence the properties of cell and their body functions (Halliwell and Gutteridge, 1985).

Although DNA is a stable, well-protected molecule, ROS can interact with it and cause several types of damage such as modification of DNA bases, single and double strand DNA breaks, loss of purines (apurinic sites), damage to the deoxyribose sugar, DNA-protein cross-linkage and damage to the DNA repair system (Barker *et al*., 2005; Repetto *et al*., 2012). The mechanism of DNA damage by peroxynitrate and hydroxyl radical include strand breaks, base pair mutations, deletions, insertions, nicking and sequence amplifications (Takane *et al*., 2002). Not all ROS can cause DNA damage and $^{\cdot}OH$ radical is one of the potential inducers of DNA damage. A variety of adducts are formed on reaction of $^{\cdot}OH$ radical with DNA. The $^{\cdot}OH$ radical can attack purine and pyrimidine bases to form $^{\cdot}OH$ radical adducts, which are both oxidizing and reducing in nature. (Kunwar and Priyadarsini, 2011).

2.10.1 Role of Antioxidants in Tissue Injury

Free radicals are constantly generated by cells, attacking their vulnerable outer membranes and eventually causing cellular degeneration and death (Hamid *et al*., 2010). Free radicals are also known to cause defects in normal RNA and also in life perpetuating DNA, which is the genetic material of the cell (Warner *et al*., 2004). It was discovered that treatment of cells with antioxidants like vitamin E blocked the usual programmed cell death cycles, allowing the cells to survive by switching their normal metabolism from the use of glucose for fuel to the use of fatty acids (Hamid *et al*., 2010). Oxidative stress has been implicated as a source of tissue injury (Powers and Jackson, 2008). The destructive effects of free radicals are prevented with the addition of antioxidants in the diet or by antioxidant supplements; a good antioxidant complex has many different specific types of antioxidants which destroy free radicals at various cellular sites (Hamid *et al*., 2010).

2.10.2 Mechanism of Action of Antioxidants

Antioxidants are involved in fundamental metabolic and homeostatic processes (Balz, 1999). According to Powers and Jackson (2008), antioxidants are substances that delay or prevent the oxidation of a substrate. In order to protect against oxidative stress, a well organized system of antioxidants works in a coordinated fashion to resist redox disturbances in cells (Powers and Jackson, 2008). Natural antioxidant system comprises of two major groups, enzymatic and non- enzymatic. Enzymatic antioxidants comprise limited number of proteins such as catalase, glutathione peroxidase and superoxide dismutase (SOD) along with some supporting enzymes. Non-enzymatic antioxidants include direct acting antioxidants, which are extremely important in defence against oxidative stress (Sherki *et*

al, 2001). Antioxidants donate their electrons to reactive oxygen species (ROS), thereby neutralizing their adverse effects (Kunwar *et al*., 2009). According to Kohen and Nyska (2002), antioxidants may function at three different levels, which include:- Prevention, that is, keeping the formation of ROS to a minimum such as desferrioxamine; interception; that is scavenging ROS either by using catalytic or noncatalytic molecules such as ascorbic acid and alpha-tocopherol and repairing of damaged target molecules such as glutathione.

Several strategies used to protect against ROS toxicity include:- Conversion of ROS into less active molecules and preventing their transformation to less active species, more deleterious forms. Antioxidants with this mechanism of action include catalase; the second mechanism of action involves minimizing the availability of prooxidants such as iron or copper ions through the use of metal binding proteins; Agents such as glutathione, uric acid and bilirubin exert their antioxidant effect via this mechanism (Powers and Jackson, 2008).

2.10.3 Antioxidant Vitamins

Antioxidant substances may be enzymatic, non-enzymatic or dietary supplements (Powers and Jackson, 2008). They are also classified according to their mode of action, location and solubility (Sharma and Sharma, 2011). Antioxidants may also be classified as primary or natural antioxidants or secondary or synthetic antioxidants (Hamid *et al*., 2010). According to Hurrell (2003), primary or natural antioxidants are mainly phenolic structures which include antioxidant minerals (selenium, copper, iron, zinc and manganese), antioxidant vitamins (vitamin C, vitamin E and vitamin B), and phytochemicals (flavonoids, catechins, carotenoids, beta-carotene, lycopene, herbs and spices). The secondary or synthetic

antioxidants are phenolic compounds, which scavenge free radicals and terminate the chain reaction. Such compounds include; butylated hydroxyl anisole, butylated hydroxyrotoluene, propylgallate and metal chelating agent (Ethylene diaamine-tetra acetic acid), tertiary butyl hydroquinone and nordihydroguaretic acid (NDGA) (Chung, 1999; Shylaja and Peter, 2004). Antioxidants are abundant in fruits, vegetables, and other foods, including nuts, grains, some meats, poultry and fish (Borek, 1991; Stevenson, 2012). Mechanistic investigation has revealed that antioxidants may exhibit prooxidant activity, depending on the specific set of conditions such as the dosage, redox conditions and the presence of free transition metals in cellular milleu (Galati *et al.*, 2002).

2.10.3.1 *Vitamin C*

Vitamin C, also known as ascorbic acid, is a monosaccharide antioxidant found in both animals and plants (Ortega, 2006). It is a water-soluble natural vitamin (Kohen and Nyska, 2002), and is, therefore, hydrophilic in nature. Thus, it functions better in aqueous environment (Yu, 1994). Vitamin C directly scavenges superoxide, hydroxyl, and lipid hydroperoxide radicals (Chelikani *et al*., 2004). It also plays an important role in regenerating vitamin E (Kohen and Nyska, 2002), a process that results in the formation of vitamin C (semiascorbyl) radical (Powers and Jackson, 2008). It is conceived that the administration of vitamin C in the form of supplements into the body may augment the function of endogenous free-radical scavengers such as superoxide dismutase, catalase and glutathione peroxidase (Yarube and Ayo, 2010).

2.10.3.2 *Vitamin A*

Vitamin A (β-carotene) is a lipid soluble vitamin and, like other carotenoids, plays an important role in the protection of cells from oxidative damage due to their highly conjugated double systems (Yarube, 2011). Carotenoids are located primarily in the membranes of tissues (El-Agamey *et al*., 2004). They are efficient biological antioxidants against lipid peroxidation because of their location and radical-scavenging capacity (Krinsky, 1998).

2.10.3.3 *Vitamin E*

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols (Janero, 1991; Schaffer *et al*, 2005; Roberts *et al*., 2007; Hamid *et al*., 2010), which are fat-soluble vitamins possessing antioxidant properties (Herrera and Barbas, 2001). Alpha-tocopherol is the best known among these collections and it possesses the most antioxidant activity (Janero, 1991; Cook et al., 2007; Abd El-Aaal, 2012). It is synthesized by plants (Basu and Dickerson, 1996), thus, it is found primarily in plant products with the richest source being plant oils (Yarube, 2011). It is found naturally in many foods such as almonds, mangoes, nuts, broccoli and in many oils including wheat germ oil, safflower, corn and soybean oils (Herrera and Barbas, 2001; Hamid *et al*., 2010). As a dietary supplement, vitamin E is available in the form of vitamin E acetate and succinate derivatives. This is because they have increased stability in air, but they are not active antioxidants until esterification at the C-6 position is reversed and the free phenol (-OH) is regenerated (Kline *et al*., 2001).

Vitamin E is one of the most widely distributed antioxidants in nature, and it is the primary chain-breaking antioxidant present in cell membranes (Parker, 1991; Schaffer *et al*, 2005; Osfor *et al*., 2010). It is a potent antioxidant (Yarube *et al.,* 2009) that scavenges ROS to maintain cellular integrity (Rai *et al.,* 2009). It has been suggested as a universal stabilizer of biological membranes in normal oxygen metabolism (Evstigneeva *et al.,* 1998). Due to its antioxidant nature, it is conceived that its administration in the form of vitamin E supplements into the body may augment the function of endogenous free-radical scavengers such as superoxide dismutase, catalase and glutathione peroxidase (Suteu *et al.,* 2007; Yarube and Ayo, 2010), thereby reducing the deleterious effects of ethanol or alcohol generally (Suteu *et al.,* 2007). Vitamin E supplementation has been shown to significantly lower lipid peroxidation through increased glutathione levels (Sushil *et al.,* 2000). Vitamin E is involved in nucleic acid and protein metabolism, functions of the mitochondria and regulation of hormone production and it is required for normal functions of the immune system and control of aggregation of platelets (Yarube, 2011). Non-antioxidant functions of vitamin E include regulation of protein kinase C, modification of cell growth and proliferation and modification of gene transcription and expression (Azzi and Stocker, 2000).

Vitamin E deficiency is rare, but may occur in people with some genetic disorders and in low-weight premature infants. Vitamin E is also used to treat cataracts, asthma, respiratory infections, skin disorders, ageing skin, sunburns, cystic fibrosis, infertility, impotence, chronic fatigue syndrome , peptic ulcers and to prevent allergies (Basu and Dickerson, 1996). Vitamin E supplementation sometimes results in increased performance and

immunity and improved oxidative status (Fragou *et al.,* 2004; Lauridsen and Jensen, 2005). Vitamin E has been shown to be effective against ethanol-induced ulcers through antioxidant action thereby providing protection (Jaarin *et al*., 2002). The use of vitamin E has also been shown to prevent gastric ulceration and oxidative stress (Fesharaki *et al*., 2006; Dawud *et al*., 2014) .

2.11 TRACE ELEMENT ANTIOXIDANTS

Antioxidant trace elements are usually obtained from diet and they are beneficial because of their ability to scavenge and neutralize ROS (Halliwell and Gutteridge, 1985; Tenorio Rodriguez *et al.,* 2013). Excessive reactive oxygen species production and low antioxidant defence may cause oxidative damage to biomolecules such as lipids, proteins and DNA (Radák *et al.*, 2005; Valko *et al.*, 2007). Antioxidants may reduce ROS production by scavenging free radicals through various mechanisms (Halliwell and Gutteridge, 1985; Vertuani *et al*., 2004). Some trace elements contribute to the function of endogenous antioxidant enzymes by acting as co-factors (Powell, 2000; Michalak, 2006) such as Cu-Zn superoxide dismutase and glutathione peroxidase activities, respectively (Machefer *et al.*, 2007), but the main antioxidant diets are the antioxidant vitamins such as vitamin E, vitamin C and β-carotene. The vitamins and their precursors neutralize several ROS (Powers *et al*., 2004) and also play a major role in exercise-induced oxidative stress (Yu, 1994). Most polyphenols act as chain-breakers or radical scavengers and prevent the oxidation of low density lipoproteins (Michalak, 2006; Gharras, 2009). Trace elements such as Fe, Cu,Se and zinc are considered essential for biological processes, including growth, reproduction, hormone metabolism and antioxidant defense (Opara and Rockway, 2006;

Tenorio-Rodriguez *et al.*, 2013) . Clinical and epidemiological research suggested a potential protective effect of antioxidant nutrients such as beta-carotene, vitamin C, vitamin E, selenium and zinc on the risk of cancer and cardiovascular diseases (Preziosi *et al.*, 1998). In a new paradigm called pharmaco-nutrition, certain key nutrients are used to modulate the inflammatory cascades caused by critical illness and protect against oxidative stress (Dupertuis *et al.*, 2009).

2.11.1 Zinc

Zinc is a micronutrient, an essential trace element for humans, animals and plants (Shah and Sachdev, 2001; Das and Das, 2012). It is involved in numerous aspects of cellular metabolism and is required for the catalytic activity of several enzymes (Anon., 2001). Zinc is not an antioxidant but may limit oxidant-induced damages in several ways (Disilvestro, 2000), some of which include protection against vitamin E depletion (Noh and Koo, 1998), stabilization of membrane structure (Bray and Bettger, 1990) and restriction of endogenous free radical production (Disilvestro, 2000). Zinc plays a major role in immune function (Prasad *et al.,* 1997), protein synthesis, wound healing and DNA synthesis (Lansdown *et al.*2007). Zinc also plays a role in cell division and growth (Prasad, 1995). The primary source of zinc is food. Some of the major sources include red meat, poultry, seafood, whole cereals and dairy products, but it is most available to the body from meat (Das and Das, 2012).

2.11.2 The Biological Role of Zinc

About ten percent (10%) of human proteins have been estimated to bind with zinc and a few hundred in addition are involved in transport of zinc (Osredkar and Sustar, 2011). Zinc is

required for proper sense of taste and smell, and it also supports normal growth during pregnancy, childhood and adolescence (Anon, 2001; Maret and Sandstead, 2006). Zinc possesses antioxidant properties, which protect cells against aging and helps speed up healing process after injury (Milbury and Richer, 2008; Osredkar and Sustar, 2011). Gastro-enteritis is strongly attenuated by ingestion of zinc, and this effect may be attributed to the antimicrobial action of zinc ions in the gastro-intestinal tract. These antimicrobial properties are visible even at low concentrations (McCarthy *et al.*1992; Rink and Gabriel, 2000). Zinc signaling is used as a way of communicating with other cells as observed in cells in the salivary gland, prostate, immune system and intestine (Hershfinkel *et al.,* 2007). Zinc may also be a neurotoxin, which suggests that its homeostasis plays a critical role in the normal function of the brain and central nervous system (Bitanihirwe and Cunningham, 2009). Zinc is bound to and transported by albumin and transferrin which also transports iron; thus, excessive iron may reduce zinc absorption and vice- versa (Valko *et al.,* 2005; Whitney and Rolfes, 2010). Blood plasma concentration remains relatively constant irrespective of the zinc intake (Anon, 2001). Metallothioneins in intestinal cells are capable of adjusting absorption of zinc by 15-40% (Aydemir *et al.,* 2006). Metallothioneins absorb both zinc and copper, thus excess zinc impairs copper absorption (Fosmire, 1990). Zinc ions are coordinated to the amino acid side chains of aspartic acid, glutamic acid, cysteine and histidine (Brandt *et al.,* 2009). Zinc as a metal also has a flexible coordination geometry, which allows proteins using it to rapidly shift conformations to perform biological reactions (Stipanuk, 2006). In general, the functions of zinc are numerous, but may be divided into three main categories, which are: catalytic, regulatory and structural roles (Stipanuk, 2006).

2.11.3 Sources of Zinc

Zinc is obtained from food sources or dietary supplements (Osredkar and Sustar, 2011). Food sources include oysters, redmeat, organ meats for example liver, beans, nuts, seafood such as crab and lobster, whole grains, cereals, almonds, pumpkin seeds and sunflower seeds. Natural zinc sources include bran, grains and fodder yeast (Berdanier *et al.,* 2007; Strnadova *et al.*, 2011). Dietary supplements may be in different forms, which are acceptable by the regulatory authorities and they include: zinc gluconate, zinc sulphate monohydrate; zinc sulphate heptahydrate, zinc carbonate, chelated zinc and amino acids, zinc acetate and dehydrate. Others are zinc chloride, monohydrate; zinc oxide, zinc lactate, trihydrate; and zinc propionate by which the percentage of elemental zinc varies by form of supplement (Disilvestro, 2004; Strnadova *et al*., 2011), although the absorption, bioavailability or tolerability of the different forms have not been determined (Maret and Sandstead, 2006). The recommended dietary intake for zinc varies with age with a tolerable upper intake for adults set at 40 mg/day.

2.11.4 Zinc Deficiency and Risk Factors

Zinc plays an important role in biological functioning of the body and its deficiency has adverse effects on the human health (Das and Das, 2012). Deficiency of zinc leads to retardation of growth and development, delayed wound healing, alopecia, poor pregnancy outcomes with teratogenic effects and decreased immune function. As a result, increased susceptibility to disease may occur (Maret and Sandstead, 2006). More than 400,000 children die every year due to zinc deficiency in developing countries (Shah and Sachdev, 2001). This indicates that about one-third of the world's population lives in countries,

where the risk of zinc deficiency is high (WHO, 2004). The WHO advocates zinc supplementation for severe malnutrition and diarrhoea (WHO, 2004). Severe zinc deficiency depresses immune function (Shankar and Prasad, 1998) and mild to moderate degrees of zinc deficiency may impair macrophage and neutrophil functions and natural killer cell activity (Rink and Gabriel, 2000). Groups at risk of zinc deficiency include pregnant and lactating mothers, breast-fed infants, people with gastro-intestinal diseases, vegetarians, and people with sickle cell disease, chronic diarrhoea, alcoholics and acroder mastitis enteropathica (Osredkar and Sustar, 2011). Factors that have effect on zinc metabolism and, thus, causing zinc deficiency include inadequate intake (low zinc-containing diets, intravenous alimentation), malabsorption (malabsorption syndrome, drugs with chelating activity), excess loss (loss into digestive fluid due to diarrhoea, increased urinary elimination with diabetes mellitus, renal disease, enhanced catabolism); increased demand (pregnancy, enhanced anabolism) among others (Yanagisawa *et al*., 1999; Murase *et al.*, 2013).

2.11.5 Role of zinc in wound healing.

Zinc maintains the integrity of skin and mucosa membranes (Anderson, 1995). Clinicians often treat skin ulcers with zinc supplements (Lansdown *et al.*, 2007). It has been observed that patients with chronic leg ulcers have abnormal zinc metabolism and low serum zinc levels (Wilkinson and Hawke, 1998). Zinc is required for protein synthesis and cell growth, and, therefore, for wound healing. Zinc supplementation has been shown to decrease wound-healing time, while a zinc deficiency leads to prolonged healing of the wound. Zinc

supplementation is indicated to aid protein synthesis and cell growth following trauma, such as in burns, surgery and wounds (Senapati and Thompson, 1985).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals and Reagents

All the chemicals were of analytical grade.

3.1.2 Animals

Forty-two adult male and female Wistar rats, weighing between 150-200 g served as subjects and were obtained from Pharmacology Department, Faculty of Pharmaceutical sciences, A.B.U, Zaria. They were quarantined for two weeks before commencement of the experiment. The animals were housed in well-ventilated cages and maintained under standardized laboratory conditions. The rats were given access to commercial laboratory diet and water *ad libitum*.

3.2 METHODS

3.2.1 Animal Grouping

The rats were grouped and treated as follows:

Group I – (Control) the rats were given only 5 ml/kg body weight distilled water.

Group II – The rats in this group were given 5 ml/kg body weight of absolute ethanol only, orally by intubation.

Group III – The rats in this group were administered with the dose of 100 mg/kg body weight cimetidine (Satoh *et al*., 1983) orally for seven days, after which the rats were fasted for forty-eight hours before induction with absolute ethanol(5 ml/kg body weight) an hour before sacrifice.

Group IV – This group was given vitamin E (100 mg/kg) orally, and ethanol (5 ml/kg body weight).

Group V – This group was treated orally with zinc gluconate (50mg/kg) and ethanol (5 ml/kg body weight).

Group VI - This group was given vitamin E orally (100 mg/kg) + zinc gluconate (50mg/kg) + ethanol (5 ml/kg body weight).

Group VII - the rats in this group were given vitamin E (100 mg/kg) + zinc gluconate (50mg/kg), only.

Each group comprised of six rats, which were treated orally with zinc gluconate, vitamin E and cimetidine for seven days using dosages described above (Arafa and Sayed-Ahmed, 2003; Ambali *et al*., 2011 and Amazan *et al.,* 2012), after which they were fasted for 48 hours and ulcer was induced with absolute ethanol. One hour afterwards, the animals were sacrificed under chloroform-induced anaesthesia (Al-Harbi *et al*., 1997). The stomachs of the rats were excised, cut along the greater curvature and gently rinsed with normal saline. The excised stomachs were stretched out as much as possible on Whatman's filter paper with mucosal surface up and then examined macroscopically.

3.2.2 Gastric Ulcer-induction by Absolute Ethanol

The rats were starved for 48 hours before the commencement of experiment as described by Abdulla *et al*. (2010) but they were allowed free access to drinking water up till 2 hours before being sacrificed. Gastric ulcer was induced by orogastric intubation of absolute ethanol (5 ml/kg body weight) according to the method described by Mahmood *et al.* (2010).

3.2.3 DETERMINATION OF GASTRIC ULCER PARAMETERS

3.2.3.1 *Determination of Ulcer Index*

The gastric lesions were evaluated according to the method described by Nwafor *et al*. (1996), where the stomachs were examined macroscopically using a hand lens, and the ulcerated surfaces measured using a transparent millimeter scale ruler. The ulcer lesions (which are the elongated black-red lines parallel to the long axis of the stomach) observed were scored according to the severity of the ulcer.

The ulcer index (UI), defined as the severity of damage caused by an ulcer-inducing agent, was then calculated using the following formula:

$$UI = UA/TA \times 100$$

Where UA= Ulcer area of stomach mucosa

TA= Total area of stomach mucosa

3.2.3.2 *Determination of Preventive Index*

The preventive index (PI), which is the degree of protection offered by a treatment against ulcer-causing agent, was calculated using the formula as described by (Hano *et al*., 1976):

$$PI = [\{MUI\ control - MUI\ treated\}/MUI\ control] \times 100$$

Where MUI = mean ulcer index

3.2.3.3 *Determination of Gastric Mucus*

The mucus of the gastric wall was determined by method of Corne *et al*. (1974) as described by Al-Mofleh *et al*., 2006). The whole stomach was weighed and transferred into a solution of 0.1% w/v Alcian blue in 0.16 M sucrose solution buffered with 0.05 M Sodium acetate at pH 5. The tissue was stained for two hours after which the excess dye was rinsed by two successive rinses with 0.25 M sucrose, for fifteen minutes and subsequently for forty-five minutes. The dye complexed with the gastric mucus was then extracted with 10 ml of 0.5 M $MgCl_2$ and was shaken intermittently for one minute at thirty minutes intervals for two hours. Exactly four mililitres (4ml) of the extract was then shaken with equal volume of diethylether. The emulsion obtained was then centrifuged at 3600 rpm for 10 minutes and the absorbance of the aqueous layer was recorded at 580 nm. The quality of the dye extracted per gram of wet glandular tissue was then calculated.

3.2.4 DETERMINATION OF GASTRIC ANTIOXIDANTS

Stomachs were cut into small pieces and homogenized in ice-cold 0.05 M phosphate-buffered saline (PBS) to give a homogenate. The homogenate was then centrifuged at 600 x g for 10 minutes at 4^{o}C, following centrifugation, the supernatant (cytosolic fraction) was then carefully removed from the pellet and used directly to assay for the activities of Superoxide dismutase (SOD), Catalase (CAT), Thiobarbituric acid reactive substances (TBARS) and Glutathione peroxidase (GPx).

3.2.4.1 *Determination of Total Thiobarbituric Acid-reactive Substances*

Lipid peroxidation of the membrane of the stomach tissue was measured indirectly by the determination of total thiobarbituric acid-reactive substances (TBARS) according to the method described by Botsoglou (1994).

Principle

Malondialdehyde (MDA) is a low molecular weight end-product of lipid hydroperoxide decomposition and is the most often measured as an index of lipid peroxidation. The assay is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA adducts which absorbs strongly at 532 nm.

Procedure

In this method, samples were incubated with 0.01 ml butylated hydroxytoluene (BHT) in ethanol, 0.025 ml phosphoric acid, in phosphate buffer (pH 7.0) with EDTA and a solution of 0.025 ml thiobarbituric acid. Samples were shaken vigorously and heated at 60°C for 60 minutes, after which they were centrifuged at 10,000 x g for 3 minutes. The red pigments in the supernatant fractions were estimated by absorbance at 532 nm.

Calculations

A calibration curve was prepared with an MDA standard (North West Life Science Specialties™, Vancouver, USA). Results were expressed as µmol MDA /mg tissue. The measure of gastric concentration of TBARS was expressed in terms of MDA as an index of lipid peroxidation.

Molar extinction of MDA is 1.56×10^5 $cm^{-1}m^{-1}$

MDA concentration = Optical Density/1.56×10^5 $cm^{-1}m^{-1}$

TBARS concentration = [Volume of homogenate x MDA concentration]/ weight of tissue

3.2.4.2 *Superoxide Dismutase Activity*

Gastric activity of SOD will be assessed according to the method of Martin *et al*., (1987).

Principle

The principle is based on the ability of superoxide dismutase to inhibit auto-oxidation of haematoxylin.

Procedure

Reaction mixture containing 0.092 ml of assay buffer (0.05M Phospahte buffer and 0.1 mM EDTA at pH of 7.5), and 0.04 ml of sample was incubated for two minutes after which 0.04 ml of haematoxylin (5 mM) was added, the changes in absorbance was recorded every ten seconds for an interval of five minutes at a wavelength of 560 nm. Enzymatic activity was expressed as Ug^{-1} tissue. One unit of SOD activity is defined as the amount of enzyme that will inhibit the rate of cytochrome c reduction by half (50%) under specific conditions.

3.2.4.3 *Catalase activity*

Catalase activity was assayed by the method of Beers and Sizer (1952).

Principle

The principle is based on the consumption of hydrogen peroxide (H_2O_2) substrate at 240 nm by catalase enzyme.

Procedure

Reaction mixture containing 0.05 ml sample and 0.095 ml assay mixture containing 0.045 ml phosphate buffer (0.01M), and 0.05ml hydrogen peroxide (0.2M) was shaken and the decomposition of hydrogen peroxide was followed by measuring the absorbance for fifteen seconds at 240 nm. The enzyme activity was expressed as Ug^{-1} tissue. One unit of catalase activity is defined as the amount of enzyme that will decompose 1.0 μMole hydrogen peroxide substrate per minute at pH 7.0 and at 25°C.

3.2.4.4 *Glutathione Peroxidase Activity*

Glutathione peroxidase activity would be carried out by method of Paglia and Valentine (1967).

Principle

The principle behind the assay of glutathione peroxidase activity is based on the ability of glutathione peroxidase to catalyse the reduction of hydrogen peroxide (H_2O_2) and oxidation of reduced glutathione (GSH) to form oxidized glutathione (GSSG) which in turn is reduced by glutathione reductase and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming $NADP^+$ which results in a reduction in absorbance at 340 nm.

Procedure

Reaction mixture containing 0.05 ml sample, 0.05 ml working NADPH (0.04 M) and 0.05 ml of 3% H_2O_2 was incubated for one minute, after which the activity was monitored for five minutes at thirty seconds interval. The glutathione peroxidase activity was then calculated and the enzyme activity was expressed as Ug^{-1} tissue. Where one unit of glutathione peroxidase is the amount of enzyme necessary to catalyze the oxidation (by H_2O_2) of 1.0 μmole NADPH to $NADP^+$, per minute at 25 ºC and pH 7.0.

3.2.5 HAEMATOLOGICAL ASSAYS

3.2.5.1 *Determination of Packed Cell Volume*

Packed cell volume (PCV) was determined using the microhaematocrit method as described by Schalm *et al.* (1975).

Procedure

A volume of uncoagulated blood was collected using heparinized capillary tubes and was centrifuged at high speed of 3000 x g for 10 minutes. The PCV values were read directly from the microhaematocrit reader and the reading was expressed as a percentage.

3.2.5.2 *Erythrocyte Indices*

Assay for erythrocyte indices such as Haemoglobin concentration (Hb), Red Blood Cell (RBC) and White Blood Cell (WBC) counts, Mean Corpuscular Volume (MCV) was carried out using the automated analyzer (SYSMEX K2XIN) which operates on the

principle of the cyanomethaemoglobin method of Alexander and Griffins (1993) and light intensity for the differential cell count.

3.2.5.3 *Evaluation of Erythrocyte Osmotic Fragility*

Erythrocyte osmotic fragility was evaluated i*n vitro* in all the rats in each group using the method described by Faulkner and King (1970), using different amounts of sodium chloride (pH 7.4) from 0.0, 0.1, 0.3, 0.5, 0.7 and 0.9 g/L in distilled water. Freshly obtained whole blood from each rat was pipetted into the test tubes, containing varying concentrations of NaCl; and then followed by careful, gentle mixing and incubation for 30 minutes at room temperature (26-28^{O}C). The samples were then centrifuged at 600 x g for 10 minutes using a bench centrifuge. The supernatant was transferred into a glass cuvette and the absorbance of the supernatant measured colorimetrically at 540 nm. The percentage haemolysis for each sample was calculated based on the following formula;

Percentage haemolysis = [O.D. of test solution/O.D. of standard solution] x 100

Where O.D. is the optical density measured

3.2.6 Histological Evaluation of Gastric Lesions

Histological evaluation was carried out by method of Culling, (1974). Specimens of the gastric walls of each rat were fixed in 10% buffered formalin and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 μm and stained with hematoxylin and eosin for histological evaluation and viewed at a magnification of x 100.

3.3 Data Analysis

The obtained data were analyzed using the analysis of variance (ANOVA) followed by DUNCAN multiple range test. The results were expressed as mean ± standard error of mean (mean ± SEM), except where otherwise stated. Values of P less than 0.05 ($P < 0.05$) was considered as significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Protective Effect of Zinc and Vitamin E on Biochemical and Physiological Changes on the Gastric Mucosa of Ethanol-induced Ulcerogenic Rats

4.1.1 Effects of Zinc and Vitamin E on Ulcer Index and Gastric Mucus of the Gastric Mucosa of Ethanol-induced Ulcerogenic Rats

The ulcer index (Table 4.1) obtained following the induction of ulcer by ethanol in the negative control group was 11.9 ± 2.83mm, and the index was significantly ($P < 0.05$) reduced to 6.0 ± 0.63mm and 0.9 ± 0.11mm in rats administered with zinc and ethanol alone and zinc, vitamin E and ethanol combined, respectively. The index was lower in zinc-treated ulcerogenic rats, when compared respectively to the index obtained in ethanol-induced ulcerogenic rats. In cimetidine-treated rats, the index was significantly ($P < 0.05$) higher than that recorded in zinc-treated ulcerogenic rats or zinc, vitamin E and ethanol-treated rats. Vitamin E showed the highest ulcer index in ulcerogenic rats when compared respectively to either cimetidine group or any other group.

The result of the gastric mucus of the stomach of the rats expressed in terms of mg dye absorbed per g of tissue is presented in Table 4.1. Again, zinc-treated rats had a higher value of gastric mucus (7.5 ± 0.10 mg dye absorbed/g of tissue) than in ulcerogenic rats treated with vitamin E alone (2.7 ± 0.40 mg dye absorbed/g of tissue) or the combination of zinc and vitamin E (6.9 ± 0.50 mg dye/g of tissue) or cimetidine (3.6 ± 0.10 mg dye /g of tissue). The gastric mucus value obtained in ulcerogenic rats treated with a combination of zinc plus vitamin E (6.9 ± 0.50 mg dye/g of tissue) did not differ ($P < 0.05$), when

compared with ulcerogenic rats treated with zinc alone (7.5 ± 0.10 mg dye/g of tissue). Treatment of the ulcerogenic rats with vitamin E alone gave the lowest gastric mucus value of 2.7 ± 0.40, and this value was significantly ($P < 0.05$) the lowest, when compared to the value recorded in any other group. The highest gastric mucus value was obtained in zinc plus vitamin E treated normal group with 12.3 ± 1.30 mg dye/g of tissue, and this value was significantly ($P < 0.05$) higher than any other value of gastric mucus obtained in the groups, including the normal control group which had gastric mucus value of 5.5 ± 0.90 mg dye/g of tissue.

The preventive index, expressed as a percentage being a measure of the efficacy of the supplements used in comparison with the mean ulcer index of the negative control (Ethanol),is presented in Table 4.1. The highest preventive ratio of 92.44% was recorded in rats administered with both zinc and vitamin E, and this value was closely followed by 89.92% recorded in ulcerogenic rats treated with zinc. The least preventive ratio of 49.58% was obtained in ulcerogenic rats administered with vitamin E.

Table 4.1: Effect of Zinc, Vitamin E and their Combination on Gastric Ulcer Index and Gastric Mucus of Ethanol induced Ulcerogenic Rats.

	Groups						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Ulcer Index (mm)	0.00 ± 0.00^{a}	11.9 ± 2.83^{c}	3.2 ± 0.29^{ab}	6.0 ± 0.63^{b}	1.2 ± 0.20^{a}	0.9 ± 0.11^{a}	0.00 ± 0.00^{a}
Gastric Mucus (mg dye absorbed/g tissue)	5.5 ± 0.90^{bc}	4.0 ± 0.20^{ab}	3.6 ± 0.10^{ab}	2.7 ± 0.40^{a}	7.5 ± 0.10^{c}	6.9 ± 0.50^{c}	12.3 ± 1.30^{d}
Preventive Index (%)	-	0	73.11	49.58	89.92	92.44	-

Values are means ± SEM of Six (6) determinations

Values with different superscript along the row differ significantly ($P < 0.05$)

Group I: Normal Control Rats, Group II : Ethanol Induced Rats, Group III: Ethanol Induced Rats Pretreated with Cimetidine (100 mg/kg), Group IV:Ethanol Induced Rats Pretreated with Vitamin E (100 mg/kg), Group V: Ethanol Induced Rats Pretreated with Zn (50 mg/kg), Group VI: Ethanol Induced Rats Pretreated with a Combination of Zinc(50 mg/kg) and Vitamin E (100 mg/kg), Group VII: Normal Rats pretreated with a combination of Zn(50 mg/kg) and Vitamin E (100 mg/kg).

4.2 Changes in Haematological Parameters of Ethanol-induced Ulcerogenic Rats

4.2.1 Effects of Zinc, Vitamin E and their combination on Some Haematological Parameters of Ethanol-induced Ulcerogenic Rats

Table 4.2 shows the variations in haematological parameters in the ethanol-induced ulcerogenic rats. There was a significant ($P < 0.05$) increase in the PCV value of the negative control group (56.2 ± 3.01 %), compared to the normal control group (43.65 ± 3.41 %) and all the other treatment groups. The group treated with vitamin E alone had the highest value (53.03 ± 5.09 %) among the treatment groups, while the lowest value of 42.97 ± 6.24 % was obtained in the zinc and vitamin E group.

The haemoglobin concentration was significantly ($P < 0.05$) different between the negative control group (17.62 ± 0.76 g/dl) and the normal control group, with a value of 14.14 ± 1.23 g/dl. There was no significant ($P > 0.05$) difference between the normal control group and the groups treated with zinc and/or vitamin E, with the group treated with vitamin E having the highest value of 17.44 ± 1.70 g/dl. The least value of 14.42 ± 0.82 g/dl was recorded in the group treated with zinc plus vitamin E, without induction of ulcer.

The highest RBC count of 10.22 ± 0.71 x 10^6/µl was recorded in the negative control group, compared to all other groups; and the value was significantly ($P < 0.05$) different from 7.08 ± 0.61 x 10^6/µl obtained from the normal control group. There was no significant ($P > 0.05$) difference between the RBC counts obtained in the normal control group, and those of all other treatment groups. The lowest RBC count of 6.56 ± 0.45 x 10^6/µl was recorded in the group treated with vitamin E and zinc.

The white blood cell (WBC) count showed significant ($P < 0.05$) difference between the negative control group ($17.7 \pm 1.7 \times 10^3/\mu l$), the normal control group ($6.1 \pm 1.3 \times 10^3/\mu l$) and the group treated with zinc and vitamin E ($12.1 \pm 2.5 \times 10^3/\mu l$), which served as the antioxidant control group. There was no significant difference between the cimetidine, vitamin E and zinc-treated groups, when compared to the normal control group. The group treated with zinc, vitamin E and ethanol had the highest value ($9.03 \pm 1.3 \times 10^3/\mu l$), while the group treated with vitamin E alone gave the least value ($5.8 \pm 1.2 \times 10^3/\mu l$).

The MCV, which is the average volume of a single cell expressed in femolitre, showed significant ($P < 0.05$) difference between the treatment groups, and the normal (55.07 ± 0.64 fl) and negative control group (57.20 ± 0.96 fl). The highest value (61.63 ± 0.22 fl) was recorded in the group treated with vitamin E. The antioxidant control group had the least value (59.73 ± 0.33 fl). There was no significant ($P > 0.05$) difference in the platelet count for all the groups.

Table 4.2: Effects of Zinc, Vitamin E and their Combination on Some Haematological Parameters of Ethanol-Induced Ulcerogenic Rats.

GROUP	I	II	III	IV	V	VI	VII
PCV (%)	43.65 ± 3.41^{a}	56.22 ± 3.01^{b}	47.89 ± 1.60^{ab}	53.03 ± 5.09^{a}	45.40 ± 3.10^{a}	45.92 ± 2.79^{a}	42.97 ± 6.24^{a}
Hb (g/dl)	14.14 ± 1.23^{a}	17.62 ± 0.76^{b}	15.58 ± 0.49^{ab}	17.44 ± 1.70^{ab}	14.92 ± 1.03^{ab}	15.18 ± 0.77^{ab}	14.42 ± 0.82^{ab}
RBC (x 10^6/µl)	7.08 ± 0.61^{a}	10.22 ± 0.71^{b}	7.94 ± 0.51^{a}	7.27 ± 0.23^{a}	6.72 ± 0.34^{a}	7.43 ± 0.24^{a}	6.56 ± 0.45^{a}
WBC(x 10^3/ µl)	6.1 ± 1.3^{a}	17.7 ± 1.7^{c}	7.5 ± 1.2^{ab}	5.8 ± 1.2^{a}	7.2 ± 0.79^{a}	9.03 ± 1.3^{ab}	12.1 ± 2.5^{b}
MCV (fl)	55.07 ± 0.64^{a}	57.20 ± 0.96^{ab}	60.3 ± 1.09^{c}	61.63 ± 0.22^{c}	60.97 ± 0.73^{c}	60.63 ± 1.53^{c}	59.73 ± 0.33^{bc}
PLT (x 10^3/ µl)	475.7 ± 14.27^{a}	986.7 ± 14.69^{a}	687.0 ± 69.37^{a}	773.0 ± 23.2^{a}	675.0 ± 20.51^{a}	865.0 ± 13.67^{a}	586.7 ± 27.72^{a}

Values are means ± SEM of Six (6) determinations

Values with different superscript along the rows differ significantly ($P < 0.05$)

PCV: Packed Cell Volume, Hb: Haemoglobin Concentration, RBC: Red Blood Cell Count, WBC: White Blood Cell Count, MCV: Mean Corpuscular Volume, PLT: Platelets.

Group I: Normal Control Rats, Group II : Ethanol Induced Rats, Group III: Ethanol Induced Rats Pretreated with Cimetidine (100 mg/kg), Group IV:Ethanol Induced Rats Pretreated with Vitamin E (100 mg/kg), Group V: Ethanol Induced Rats Pretreated with Zn (50 mg/kg), Group VI: Ethanol Induced Rats Pretreated with a Combination of Zinc (50 mg/kg) and Vitamin E (100 mg/kg), Group VII: Normal Rats pretreated with a combination of Zn (50 mg/kg) and Vitamin E (100 mg/kg).

4.3 Effects of Zinc, Vitamin E and their Combination on Osmotic Erythrocyte Fragility of Ethanol-induced Ulcerogenic Rats

The results of osmotic erythrocyte fragility are shown in Table 4.3. In general, percentage haemolysis decreased with increasing NaCl concentration, irrespective of the treatment group. Complete haemolysis (100%) occurred in the standard solvent, which is distilled water. There was a significant ($P < 0.05$) difference in the percentage haemolysis of the treated animals, when compared to the control groups. The rats treated with zinc had the highest percentage haemolysis of 7.89 ± 1.08%, followed by the group treated with vitamin E (6.86 ± 1.05%) and lastly the group treated with zinc + vitamin E (6.67 ± 0.71%). Although the values did not differ among the treatment groups, the percentage haemolysis in each treatment group was higher compared with the normal control (3.01 ± 0.43%); the result indicated that there was a significant increase in the treatment group compared to that of the control group. At 0.5% NaCl concentration, a significant ($P < 0.05$) increase was obtained in the group treated with a combination of zinc + vitamin E (43.95 ± 7.71%) as compared to the group treated with ethanol alone (24.72 ± 1.86%). Although at 0.3% NaCl concentration, there was a significant decrease in the group treated with zinc, vitamin E and ethanol (74.72 ± 3.25%), compared to the group treated with ethanol only. At the 0.1 and 0.7% concentrations, there were no significant ($P < 0.05$) changes in the percentage haemolysis among the rats in the various treatment groups.

Table 4.3: Effect of Zinc, Vitamin E and their Combination on Osmotic Erythrocyte Fragility of Ethanol-induced Ulcerogenic Rats

	Group						
Percentage Haemolysis (%)	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
0.1%	95.95 ± 0.79^{b}	95.45 ± 1.81^{ab}	94.44 ± 2.98^{ab}	87.78 ± 4.74^{a}	91.57 ± 1.05^{ab}	90.29 ± 1.05^{ab}	94.25 ± 2.11^{ab}
0.3%	83.71 ± 5.02^{ab}	90.20 ± 2.25^{b}	89.29 ± 4.51^{b}	83.43 ± 6.31^{ab}	89.80 ± 1.62^{b}	74.72 ± 3.25^{a}	94.25 ± 2.11^{b}
0.5%	24.72 ± 1.86^{ab}	13.70 ± 6.22^{a}	37.71 ± 2.36^{bc}	31.36 ± 3.28^{bc}	36.48 ± 1.25^{bc}	43.95 ± 7.71^{c}	46.86 ± 6.53^{c}
0.7%	6.24 ± 1.54^{a}	4.75 ± 0.65^{a}	3.76 ± 1.13^{a}	4.73 ± 0.76^{a}	3.76 ± 1.13^{a}	4.22 ± 0.31^{a}	5.09 ± 1.35^{a}
0.9%	3.01 ± 0.43^{a}	6.60 ± 1.74^{bc}	4.23 ± 0.13^{ab}	6.86 ± 1.05^{bc}	7.89 ± 1.08^{c}	6.67 ± 0.71^{bc}	5.84 ± 0.70^{abc}

Values are means ± SEM of Four (4) determinations

Values with different superscript along the rows differ significantly ($P < 0.05$)

Group I: Normal Control Rats, Group II : Ethanol Induced Rats, Group III: Ethanol-induced Rats Pretreated with Cimetidine (100 mg/kg), Group IV:Ethanol-induced Rats Pretreated with Vitamin E (100 mg/kg), Group V: Ethanol-induced Rats Pretreated with Zn (50 mg/kg), Group VI: Ethanol-induced Rats Pretreated with a Combination of Zinc (50 mg/kg) and Vitamin E (100 mg/kg), Group VII: Normal Rats pretreated with a combination of Zn (50 mg/kg) and Vitamin E (100 mg/kg).

4.4 *IN VIVO* ANTIOXIDANT STUDIES

4.4.1 Effects of Zinc, Vitamin E and their Combination on Lipid Peroxidation and Endogenous Antioxidant Enzymes in Ethanol-Induced Ulcerogenic Rats

The results of the activities of some tissue antioxidant enzymes and lipid peroxidation in the rats are presented in Table 4.4. There was a significant ($P < 0.05$) increase in TBARS in the negative control group (11.30 ± 0.15 μmol/g tissue) compared to that of the normal control (8.12 ± 0.35 μmol/g tissue). The group pretreated with a combination of zinc and vitamin E combined had the lowest value (8.52 ± 0.87 μmol/g tissue), followed by the group treated with zinc alone (8.77 ± 0.55 μmol/g tissue) while the highest value (10.01 ± 0.54 μmol/g tissue) was recorded in the rats treated with vitamin E alone.

For the activity of SOD, there was significant ($P < 0.05$) increase in the value (2.08 ± 0.10 U/g tissue) in the group treated with vitamin E, while for all the other groups there was no significant ($P < 0.05$) difference compared with the normal control group (1.63 ± 0.14 U/g tissue). For glutathione peroxidase activity, the group showed significant ($P < 0.05$) increase in the group treated with vitamin E alone (43.17 ± 0.38 U/g tissue) as compared with the normal control group (39.61 ± 3.18 U/g tissue) when assayed. There was no significant difference when compared with all the other groups. The result of catalase activity indicated no significant difference in the groups treated with vitamin E (49.61 ± 1.73 U/g tissue) as compared to the normal control group (48.55 ± 2.76 U/g tissue), but catalase activity in all the other groups was significantly ($P < 0.05$) different from that of the group treated with zinc, having a value of 42.64 ± 2.73 U/g tissue.

Table 4.4: Protective Effect of Zinc, Vitamin E and their Combination on Tissue Antioxidant Enzymes and Lipid Peroxidation of Ethanol-induced Ulcerogenic Rats.

	Group						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
TBARS (µmol/g tissue)	8.12 ±0.34[a]	11.30 ±0.15[b]	10.16 ±0.69[ab]	10.01±0.53[ab]	8.77 ±0.54[a]	8.52 ±.0.86[a]	9.11±1.12[a]
SOD (U/g tissue)	1.63 ± 0.14[a]	1.49 ± 0.12[a]	1.74 ± 0.08[ab]	2.08 ± 0.10[b]	1.56 ± 0.11[a]	1.58 ± 0.17[a]	1.52 ± 0.05[a]
GPx (U/g tissue)	39.61 ± 3.18[ab]	33.73 ± 1.23[a]	36.10 ± 2.63[ab]	43.17 ± 0.38[b]	37.14 ± 2.52[ab]	37.73 ± 3.37[ab]	42.47 ± 3.59[b]
CAT (U/g tissue)	48.55 ± 2.76[b]	40.58 ± 0.96[a]	49.91 ± 2.68[b]	49.61± 1.73[b]	42.64 ±2.73[ab]	42.21± 2.62[ab]	44.24 ± 2.62[ab]

Values are means ± SEM of Four (4) determinations

Values with different superscript along the rows differ significantly ($P < 0.05$)

TBARS- Thiobarbituric-acid Reactive Substances; SOD- Superoxide Dismutase; GPx- Glutathione Peroxidase; and CAT- Catalase

Group I: Normal Control Rats, Group II : Ethanol-induced Rats, Group III: Ethanol-induced Rats Pretreated with Cimetidine (100 mg/kg), Group IV:Ethanol-induced Rats Pretreated with Vitamin E (100 mg/kg), Group V: Ethanol-induced Rats Pretreated with Zn (50 mg/kg), Group VI: Ethanol-induced Rats Pretreated with a Combination of Zinc (50 mg/kg) and Vitamin E (100 mg/kg), Group VII: Normal Rats pretreated with a combination of Zn (50 mg/kg) and Vitamin E (100 mg/kg).

4.5 Effect of Administration of Zinc,Vitamin E and their combination on Histological Changes in the Gastric Mucosa of Ethanol-induced Ulcerogenic Rats.

Histological observations of the ethanol-induced gastric lesions in the ulcer control group (without treatment) showed extensive damages to the gastric mucosa. The damages included disruption of the epithelium, necrosis of the mucosa, with leucocyte infiltration of the mucosal layer (Plate 4.2). Rats treated with zinc, vitamin E and zinc plus vitamin E had comparatively better protection of the gastric mucosa as evidenced by the reduction in the ulcerated area, reduced or absence of necrosis and leucocytic infiltration (Plate 4.2 – 4.6), with plate 4.2 showing extensive damage to the apical mucosa characterized by severe damage to the epithelium with severe erosion (a), necrosis of the mucosal cells(c) and leucocyte infiltration(b) of the mucosa; and plate 4.3 showing less severe damage to the gastric mucosa, with lesser degree of erosion (a) and destruction of the surface mucosal cells and leucocyte infiltration(b); while plates 4.4 and 4.5 showed mild damage(a) to the gastric mucosa of the rats with infiltration of cells and slight necrosis(b) and plate 4.6 showing mild damage to the surface mucosal cells(a) with infiltration of cells with leucocytes(b). Plates 4.1 and 4.7 showed normal gastric mucosa without and with treatment respectively.

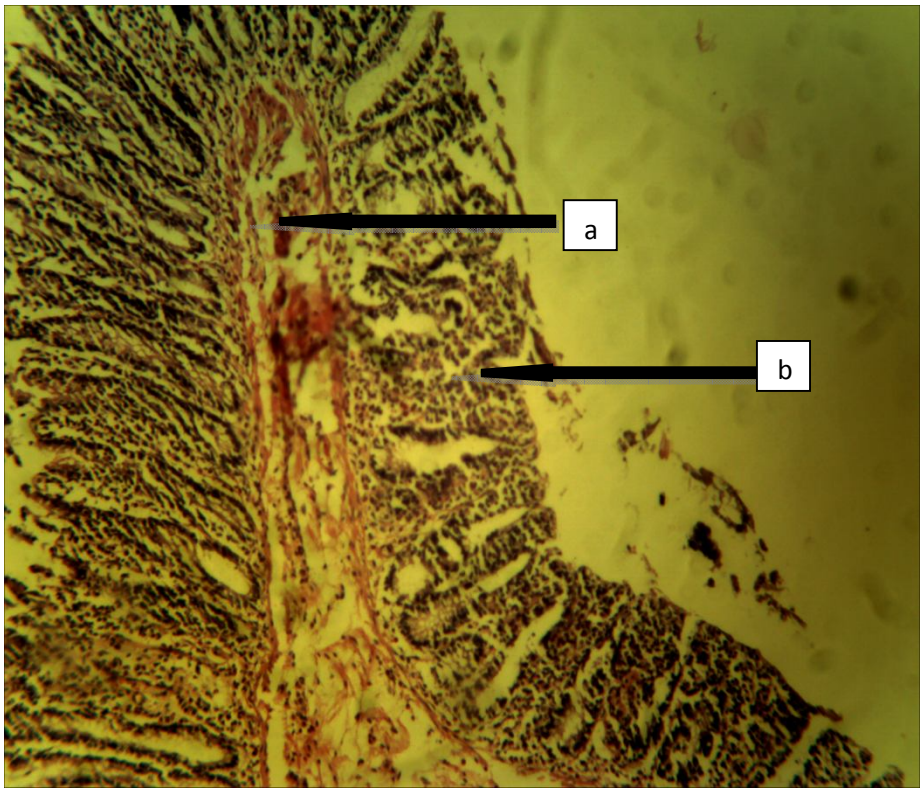


Plate 4.1: (Normal Control): Photomicrograph of the normal gastric mucosa(a) and submucosa (b) of the rats given distilled water only.

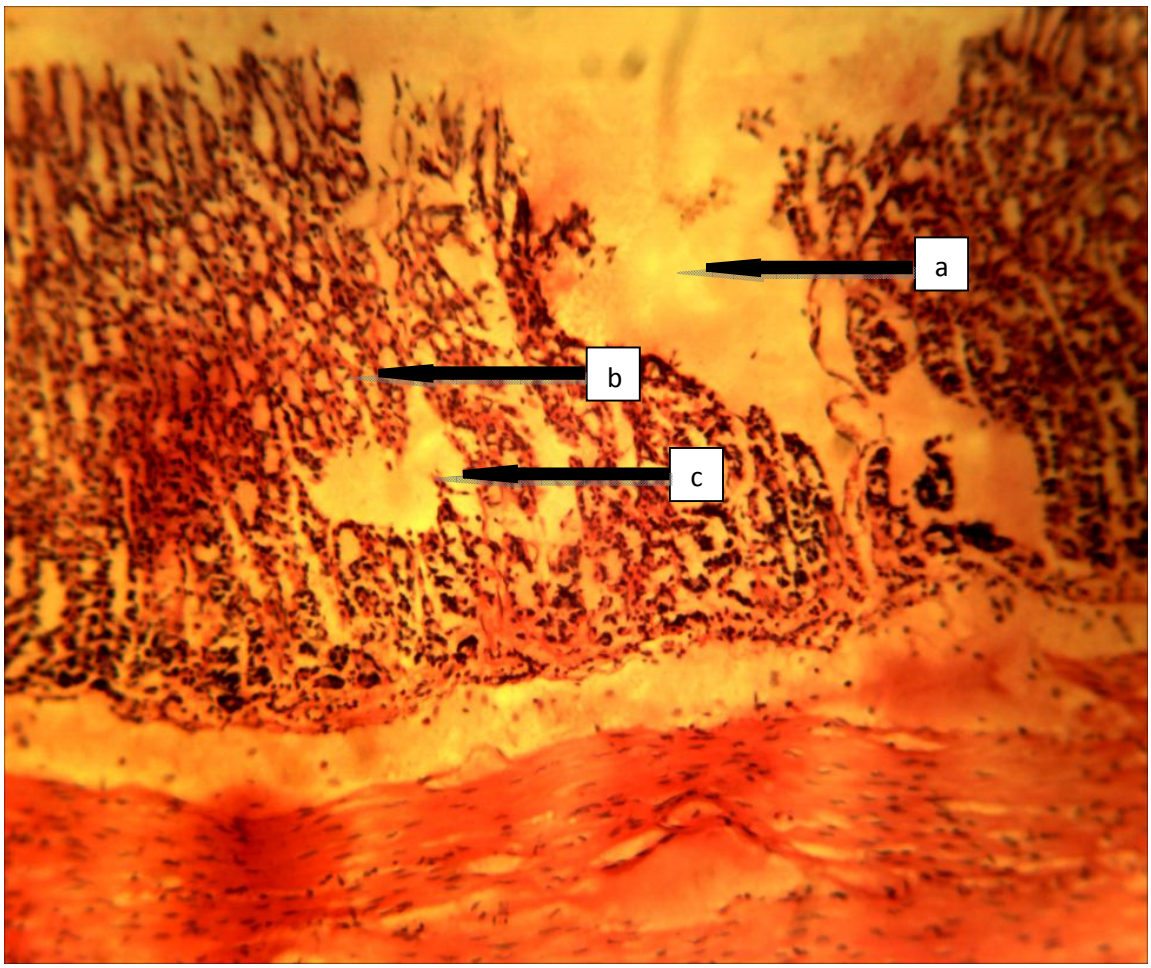


Plate 4.2:(Ethanol$_{5mg/kg}$): Photomicrograph showing extensive damage to the apical mucosa characterized by severe damage to the epithelium with severe erosion (a), necrosis of the mucosal cells(c) and leucocyte infiltration(b) of the mucosa (H and E, x 100)

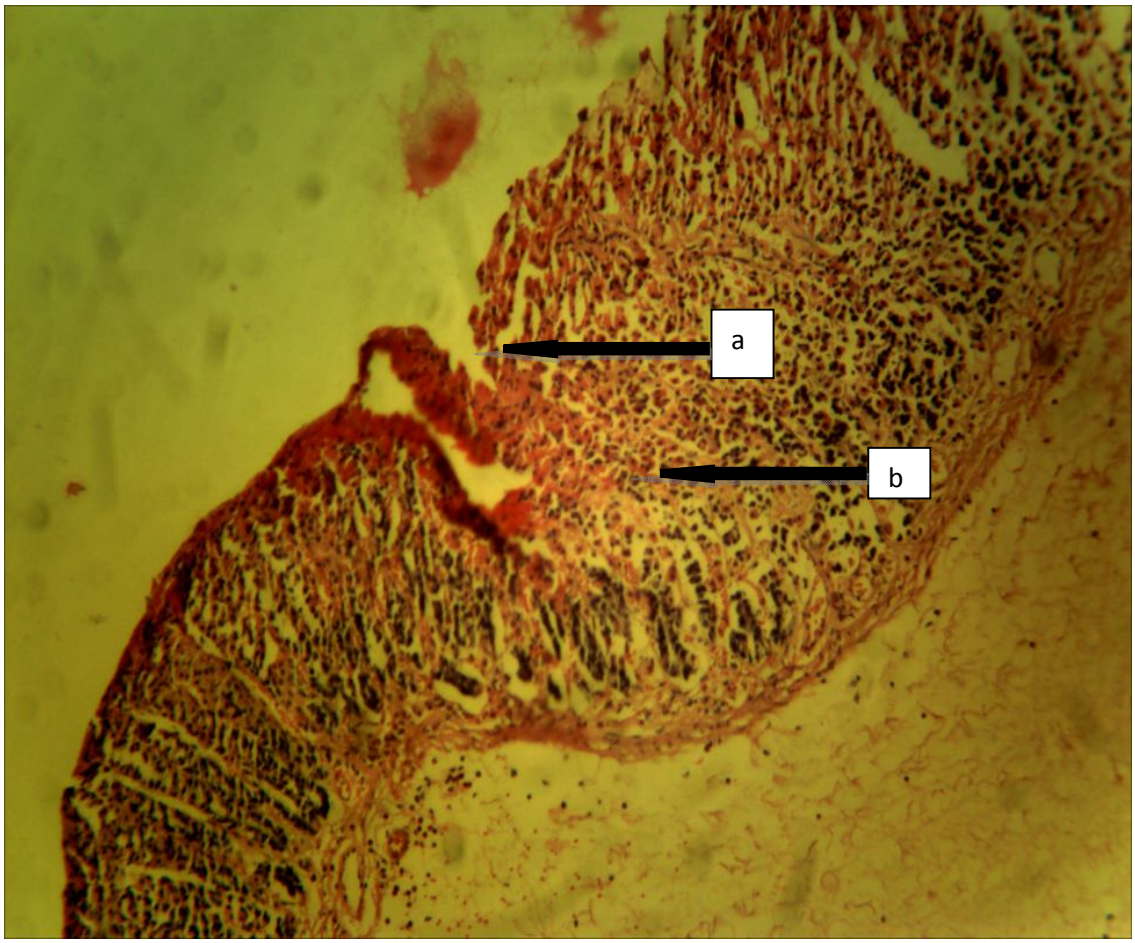


Plate 4.3: (Cimetidine$_{100\ mg/kg}$ + Ethanol$_{5mg/kg}$): photomicrograph showing less severe damage to the gastric mucosa, with lesser degree of erosion (a) and destruction of the surface mucosal cells and leucocyte infiltration (b). (H and E, x 100)

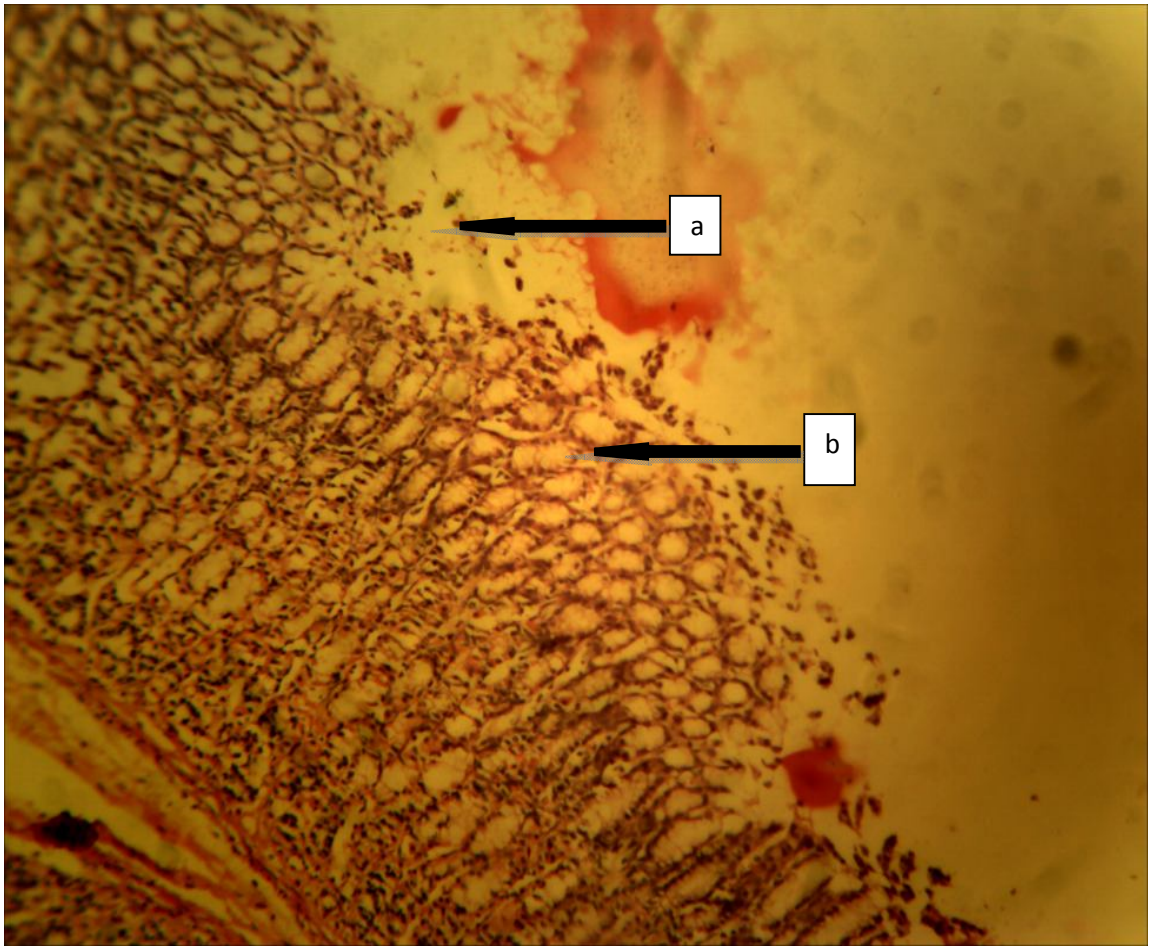


Plate 4.4: (Vitamin $E_{100mg/kg}$ + $Ethanol_{5mg/kg}$). Photomicrograph showing mild damage (a) to the gastric mucosa of the rats with infiltration of cells and slight necrosis (b). (H and E, x 100)

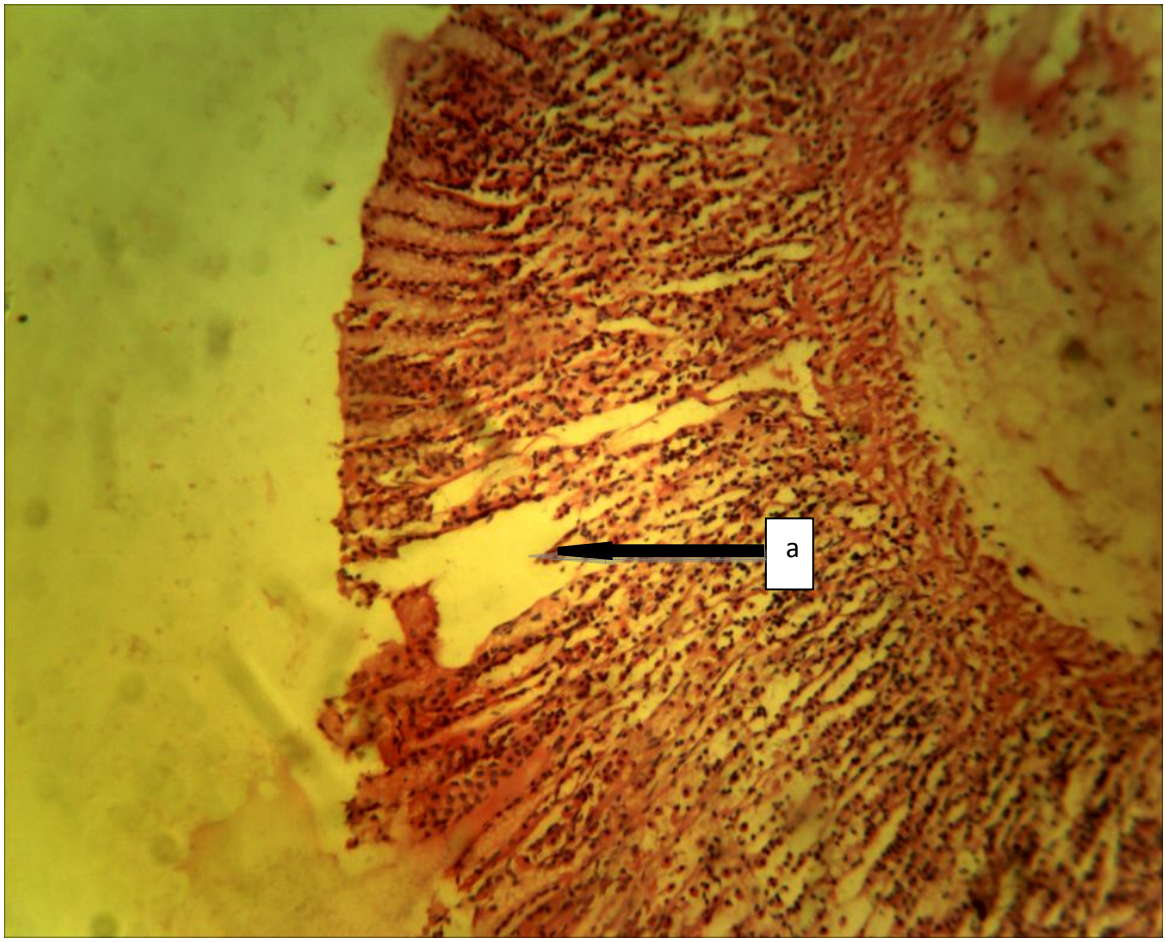


Plate 4.5: (Zinc $_{50mg/kg}$ + Ethanol$_{5mg/kg}$): Photomicrograph showing mild erosion (a) of the gastric mucosa with necrosis of cells (H and E, x 100)

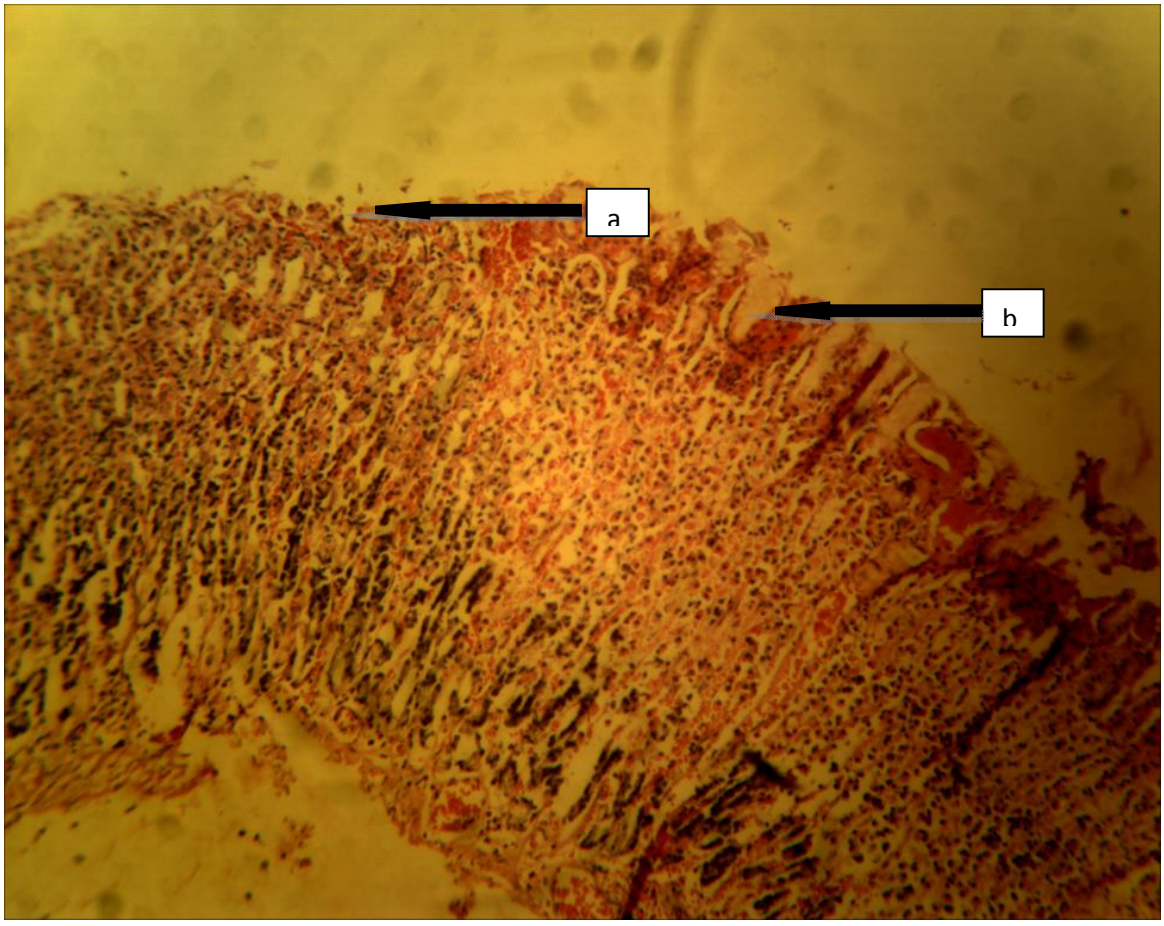


Plate 4.6: ($Zinc_{50mg/kg}$ + Vitamin $E_{100mg/kg}$ + $Ethanol_{5mg/kg}$): Photomicrograph showing mild damage to the surface mucosal cells(a) with infiltration of cells with leucocytes(b)(H and E, x 100)

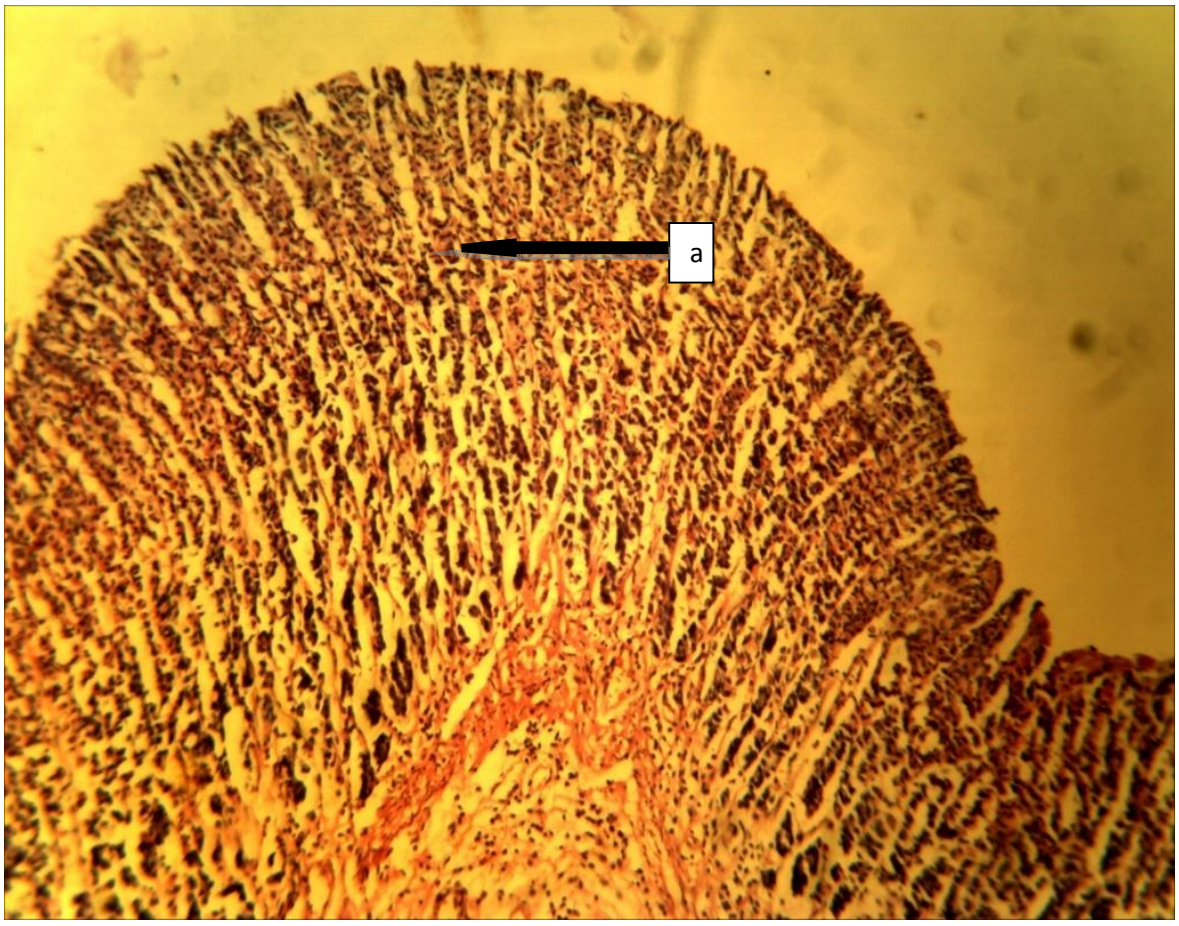


Plate 4.7: ($Zinc_{50mg/kg}$ + Vitamin $E_{100mg/kg}$): Photomicrograph showing normal mucosal layer (a) without damage (H and E, x 100).

CHAPTER FIVE

5.0 DISCUSSION

Absolute ethanol is commonly used for induction of ulcer and it leads to intense gastric mucosal damage (Al-Radahe *et al*., 2012). Previous studies suggest that the ethanol-induced injury to the gastro-intestinal mucosa starts with microvascular injury, which includes disruption of the vascular endothelium and resulting in increased vascular permeability, oedema formation and epithelial lifting (Szabo *et al*., 1995).

The result of the present study demonstrated that zinc alone was more protective against gastric mucosa damage, induced by ethanol than vitamin E alone.The protection induced by vitamin E was the least as evidenced by a higher ulcer index, compared to that of zinc. This result is in agreement with the findings of Ismail *et al.* (1999), who suggested that vitamin E accelerates healing of gastric lesions, but may not prevent the formation of ethanol-induced lesions. The findings of the present study showed that zinc alone, is more protective than both vitamin E alone and even the conventional anti-histaminic drug, cimetidine which is a H_2-receptor antagonist (H_2-blocker). The least ulcer index of 0.9 ± 0.11mm was obtained in the group treated with a combination of the two antioxidants, vitamin E and zinc. This may be as a result of the ability of both zinc and vitamin E to stabilize the membrane (Agarwal *et al*., 2011). Thus, the combination increased the potency of the action of the two antioxidants, resulting in synergestic effects. Both the normal and, in particular, the antioxidant control showed that neither the distilled water nor any of the two antioxidants administered exerted any deleterious effect on the gastric mucosa. This observation may be responsible for the lack of ulcer index (0%) obtained in both control groups.

The finding of the present study demonstrated that ROS are, apparently, involved, at least in part, in the mechanism underlying the ulcerogenesis (Iyyam Pillai *et al.,* 2010) since administration of antioxidants ameliorated the ulcer index in rats, especially a combination of vitamin E and zinc. This result is of practical application. Individuals with ulcer may, therefore, benefit from the administration of zinc and/or vitamin E and, especially, a combination of the two antioxidants.

The result of the present study demonstrated that agents acting on H_2-receptors may be partly responsible for the induction of ulcer in individuals with the disease, and it agreed with the finding of Iyyam-Pillai *et al*. (2010) that ROS also play a significant role in ulcerogenesis.

The result of the present study demonstrated that agents acting on H_2-receptors such as alcohol may be partly responsible for the induction of ulcer in individuals with the disease, and it agreed with the finding of Iyyam Pillai *et al*. (2010) that ROS also play a significant role in ulcerogenesis.

The result of the gastric mucus value and preventive ratio showed that zinc alone was more protective than vitamin E, and that a combination of zinc and vitamin E all the more gave a better protection. The finding is in agreement with the results of Banos and Bulbena (1989) and Bandyopadhyaly and Bandyopadhyaly (1997), who showed that zinc reduces acid secretion and increases mucus secretion. The result obtained in the present study has further demonstrated that zinc and/or vitamin E protect cells from injuries, particularly the

combination of the two antioxidants. The result also showed that the least preventive index and gastric mucus value was obtained in rats administered with vitamin E, which agree with the finding of Ismail *et al*. (1999), who studied the effects of palm vitamin E on ethanol-induced gastric lesions. Therefore, zinc alone or its combination with vitamin E, and not vitamin E alone, may be more therapeutic in action than vitamin E alone or even cimetidine, with gastric mucus value of 3.6 ± 0.10 mg dye/g tissue and preventive ratio of 73.11%. The result of the present study has shown that either zinc alone or its combination with vitamin E has a more potent therapeutic effect in gastric ulcer, induced by ethanol than even cimetidine, the conventional anti-histaminic agent (positive control). It is worth noting that the antioxidants which are less toxic, readily available than cimetidine gave a better therapeutic effect in ulcerogenic rats than the conventional anti-histaminic agent, cimetidine. The present study has, therefore, shown that, not only that the antioxidants are more potent, but also that their combination exert a synergestic effect.

The functional state of the blood systems changes dynamically according to the nature, strength and duration of exposure to external factors (Dimitrova *et al.,* 2010). The study indicated that groups treated with zinc and zinc + vitamin E had PCV, Hb, and RBC count closer to the normal control as compared to those treated with vitamin E alone. This result showed a significant ($P < 0.05$) decrease in the PCV, Hb and RBC count in the groups administered with cimetidine and zinc as compared to the negative control group, which is in agreement with the findings of Yadrick *et al*. (1989) and Dimitrova *et al*. (2010) that zinc supplementation decreases haematocrit value and, by implication, Hb concentration value. The result also showed that the group in which ulcer was induced without treatment had a

higher value of PCV, Hb, RBC count and WBC count as compared to the normal control group. This is contrary to the findings by Oyedeji *et al*. (2013), who obtained a significant reduction in PCV, Hb, RBC and lymphocyte counts as compared to the normal control after treatment with alcohol over a period of thirty days. In the vitamin E treatment group, there was an increase in PCV, Hb and RBC count, while the WBC count decreased compared to that of the normal control group, although the changes were not significant. The findings of the present study differed from the result obtained by Kumari *et al*. (2013), who reported that the values for haemoglobin concentration and PCV were maintained after treatment with vitamin E in a dose-dependent manner. The variation in WBC count may be as a result of antioxidant properties of the various supplements used, which have the ability to boost the immune system (Zago and Oteiza, 2001; Okwu, 2004); as evidenced by the low WBC count, obtained in the vitamin E-treatment group. The increase in RBC count may be as a result of the treatment, which apparently, increased the oxygen-carrying capacity of the blood by increasing the production of RBC in the bone marrow (Nebedum *et al*., 2010).

The erythrocyte osmotic fragility (EOF) is a measure of erythrocyte strength and its ability to withstand varying osmotic gradients (Aldrich *et al*., 2006). It is used as an indirect method of evaluating lipid peroxidation in animals (Chihuailaf *et al*., 2002; Asala *et al*., 2011; Minka and Ayo, 2013). In the present study, following exposure to ethanol, vitamin E and zinc, there was a significant ($P < 0.05$) decrease in the percentage haemolysis in groups treated with vitamin E and zinc, compared to the ulcerogenic rats without treatment at 0.3% and 0.5% NaCl concentration. This finding may be linked to the lipoperoxidative mechanism of alcohol induction of gastric mucosal damage,involving the generation of

ROS in excess amounts (Wu and Cederbaum, 2005). The structural integrity of the erythrocyte membrane is an important feature for its resistance to peroxidative attack (Gain and Shodet, 1981), since the RBCs are highly susceptible to oxidative stress as a result of high amount of unsaturated fatty acid content in their membranes (Altan *et al*., 2003; Agrawal and Sharma, 2010). Increased free-radical generation has been established to cause oxidative stress (Chakraborti *et al*., 2008; Halliwell, 2009; Nazifi *et al*., 2009). Furthermore, the administration of zinc has been demonstrated in this study to protect the RBCs significantly from ethanol-induced toxicity and, apparently, via lipid peroxidative damage. This finding is in agreement with the results of Ambali *et al*. (2010a), who showed that zinc exerts ameliorative effect against erythrocyte fragility and lipid peroxidation changes in rats exposed to chlorpyrifos poisoning. This protective effect of zinc, which was not directly investigated in the present study, may occur as a result of its antioxidant properties (Ambali *et al*., 2010a).

In this study, there was a significant increase in the MDA content as indicated in the value of the total TBARS in the ulcerogenic rats without treatment, compared to those obtained in all the other treatment groups. This result is in agreement with the consensus that the deleterious effects of ethanol on gastric mucosa occur as a consequence of enhanced lipid peroxidation (Ismail *et al*., 1999; Ligha and Fawehinmi, 2009), and also supports the findings that antioxidants protect the gastric mucosa from injury (Popovic *et al.*, 2009). Ethanol produces necrotic lesions by direct necrotizing action, which in turn reduces defensive factors such as the secretion of bicarbonate and production of mucus (Alrashdi *et al*., 2012). The pathogenesis of ethanol-induced gastric mucosal damage occurs directly and

indirectly through various mediators such as lipoxygenase, cytokines and oxygen-derived-free radicals (Abdel-Salam *et al*., 2001).

The values of SOD, catalase and GPx activity showed no significant difference among all the groups, with the exception of the group of ulcerogenic rats treated with vitamin E, which had a higher value compared to the other treatment groups. This result is in agreement with the findings of Popovic *et al*. (2009) and Selvamathy *et al*. (2010), who demonstrated that ROS, especially $^{\cdot}OH$, plays a major role in oxidative damage of gastric mucosa in almost all forms of gastric ulcer (Das *et al*., 1997). The damage caused by superoxide anions is counteracted by dismutation with SOD (Fridovich, 1986). SOD in turn converts the reactive hydrogen peroxide to water, which, if not scavenged by catalase, can cause lipid peroxidation by increasing generation of hydroxyl radicals (Das *et al*., 1997). Thus, the increased catalase activity obtained in the present study in groups administered with zinc and/or vitamin E shows that there was reduction in lipid peroxidation and tissue damage, which is in agreement with the findings obtained by Popovic *et al*. (2009) following the administration of some drugs in ethanol-induced ulcers. This also supports the findings of Maini *et al.* (2007) that vitamin E ameliorates the negative effects of oxidative stress/damage. The changes in the status of both enzymatic and non-enzymatic antioxidants in the tissue showed that there was reduced oxidative stress after treatment with antioxidants (McCardle and Jackson, 2008); in this case vitamin E and zinc supplements, compared to the ulcerogenic control group, without the treatment.

The induction of gastric ulcer by ethanol in the present study agrees with the previous findings that the alcohol produces necrotic lesions in the gastric mucosa by its direct toxic effect, by reducing the secretion of bicarbonates and production of mucus (Rujjanawate *et al*., 2005; Al-Radahe *et al*., 2012). This observation was supported by the decrease in the damage, obtained following treatment with zinc and vitamin E. Thus, oral administration of absolute ethanol is noxious to the stomach as it affects the gastric mucosa topically by disrupting its barrier and provoking pronounced microvascular changes within few minutes of administration (Moleiro *et al.*, 2009). Furthermore, the result of the present study has agreed with the findings of Jelski *et al.* (2009), who showed that ethanol produces linear haemorrhagic lesions, extensive sub-mucosal oedema, mucosal friability, inflammatory cell infiltration, and epithelial cell loss in the stomach. Thus, the supplements exerted cytoprotective effects on the gastric mucosa.

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Vitamins and trace elements in the form of nutritional supplements are of great importance in the maintainance of human health and also in the protection against a great number of diseases including gastro-intestinal disorders. The results of this study showed:

i) The protective effect of zinc, vitamin E and their combination on ethanol-induced ulcer; with the combination of zinc and vitamin E having the highest protective ratio, indicating a higher potential as a means of protection against gastric ulcer.

ii) There was amelioration of oxidative stress in the group treated with zinc and the combination of zinc and vitamin E. Lipid peroxidation reduced significantly in the treatment groups and there was increased superoxide dismutase, glutathione peroxidase and catalase activities in the vitamin E treated group, compared to the other groups.

iii) The treatments with zinc, vitamin E and their combination had positive effect on the haematological parameters by facilitating the maintainance of the normal haematological conditions compared to the untreated ulcerogenic rats.

6.2 RECOMMENDATION

We would like to recommend that:-

i) Further studies should be directed towards the mechanism by which zinc, vitamin E and their combination offer protection against ethanol-induced ulcer.

ii) Further studies should also be directed towards determining the appropriate ratio/dosage of both zinc and vitamin E to be used in order to obtain efficient protection against ethanol-induced ulcer. This is because antioxidant activity is dose dependent.

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