COMPARATIVE EFFECTS OF *Nigella sativa*SEED OIL AND ZINC GLUCONATE ONETHANOL-INDUCED GASTRIC MUCOSAL DAMAGE AND SECRETION IN WISTAR RATS

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DECLARATION

I declare that the work in this thesis, entitled, "Comparative effects of *Nigella sativa*Seed Oil and Zinc Gluconate on Ethanol-Induced Gastric Mucosal Damage and Secretion in Wistar Rats" has been carried out by me in the Department of Human Physiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at this or any other Institution

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CERTIFICATION

This thesis entitled **"**COMPARATIVE EFFECT OF *NIGELLA SATIVA* SEED OIL AND ZINC GLUCONATE ON ETHANOL-INDUCED GASTRIC MUCOSAL DAMAGE AND GASTRIC SECRETION IN WISTAR RATS"by Abubakar Musa GARBA meets the regulations governing the award of the degree of Master of Science Degree in Human Physiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to Allah (SWT), my family and the Muslim Ummah.

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ABSTRACT

A wide spread search has been launched to identify new anti-ulcer therapies from natural sources, with minimal side effects. The aim of the study was to compare the effects of *Nigella sativa*seed oil and zinc gluconate on ethanol-induced gastric mucosal damage and secretion in Wistar rats. A total of 50 male rats, weighing between 150-200g were used for the study. The animals were then sub-divided into two sub-groups of 25 rats each for gastric mucosal damage and gastric secretion studies respectively. Each of the sub-groups were divided into 5 groups of 5 rats each and treated with distilled water (10ml/kg), absoluteethanol (1ml/kg),*Nigella sativa* oil (5ml/kg), zinc gluconate (50mg/kg) and zinc gluconate (50mg/kg) plus *Nigella sativa oil* (5ml/kg) respectively. The phythochemical screening of the *Nigella sativa* oil revealed the presence of carbohydrate, unsaturated steroids, triterpenes, cardiac glycoside and flavonoids. The results of the study revealed the normal architecture of gastric mucosa for the control group with ulcer index of 0.0 ± 0.0 mm. The ethanol-treated group showed severe necrosis-of gastric epithelium,with ulcer index of 10.25± 0.85 mmas compared to the control. The *Nigella sativa* oil, zinc gluconate and Nigella sativa plus zinc gluconate treated groups have demonstrated significant decrease in the ulcer indices of 5.50 ± 1.04 mm, 4.75 ± 0.25mm and 3.75 ± 1.44mm respectively as compared to the control with 0.0 ± 0.0 mm. On the other hand the groups also showed preventive indices of 48.8%, 53.6% and 63.4% respectively when compared to those of the control 100% and ethanol treated group with no protectionWith regard to gastric juice secretion, the ethanol-treated group showed an increase in the volume of gastric juice of 1.10 ± 0.51 ml/3h, titratible acidity of 55.00 ± 19.62mEq and mean acid output of 91.30 ± 62.31µEq/h, zinc gluconate treated showed a slight increase in volume of gastric juice of 0.82 ± 0.15ml/3h,titritable acidity of 41.00 ± 7.65 mEq/l and mean acid

output of 38.30 ± 15.25 µEq/h when compared to control groupwith volume of gastric juice of 0.72 ± 0.08ml/3h, titritable acidity of 36.00±4.30 and mean acid output of 27.40 ± 6.52 µEq/h. With regard to *Nigella sativa* oil treated group,*Nigella sativa* oil plus zinc gluconate treated groups demonstrated a decrease in the volume of gastric juice of 0.56 ± 9.17 ml/3h, titritable acidity of28.00 ± 8.68 mEq/l and acid output of21.50 ± 12.85µEq/h. In conclusion,*Nigella sativa* and zinc gluconate conferred protection to gastric mucosa but the combination of the two produced an additive effect.

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ABBREVIATIONS AND SYMBOLS

GIT:	Gastrointestinal Tract
H.Pylori:	*Helicobacter Pylori*
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
GSH – Px	Reduce Glutathione Peroxides
MDA	Malonaldehyde
IRI	Ischemic Reperfusion Injury
MUC	MucinGlucoprotein
TTF	Trefoni Factor Family
TGF	Transforming Growth Factor
PGE	Prostaglandin E
MARK	Mitogen Activate and Protein
TNF	Tumour Necrotic Factor
NO	Nitric Oxide
CGRP	Calcitonin Gene Related Factor
NOS	Nitric Oxide Synthase
CRF	Corticotrophic Releasing Factor
NSAID	Non-Steroidal Anti-inflammatory Drugs
HDL	High Density Lipoprotein
ATP	Adenosine Triphosphate

ADP	Adenosine Diphosphate
AMP	Adenosine Monophosphate
PDR	Physician Desk References
CPA	Canadian Pharmacist Association
EGD	Esophagogastro-Duodenoscopy
ZTP	Zinc Transport Proteins
LDL	Low Density Lipoprotein
VLDL	Very Low Density Lipoprotein
TBARs	Thiabarbituric Acid Reactive Substances
TQ	Thymoquinonine
LD_{50}	Lethal Dose
NSO	*Nigella Sativa* Oil
NARICT	National Research Institute of Chemical Technology Zaria
GP	Gastric pit

CHAPTER ONE

1.0 Introduction

Ulcers are lesions penetrating through the thickness of gastro-intestinal tract (GIT) mucosa and *Muscularis mucosae.* The time trends in the epidemiology of peptic ulcer disease reflect complex, multi-factorial aetiologies. Peptic ulcers were rare before the 1800s. The pathology of gastric ulcers (GUs) was first described in 1835, during the late 1800s the prominent form was GUs in young women. Duodenal ulcers (DUs) were rare until about 1900, and then it became a prevalent condition during the first half of the 20th century. However, in developed countries, the mortality from peptic ulcer disease has fallen dramatically for birth cohorts born after the turn of the 20th century (Sonnerberg, 2007). It is the most common GIT disorder, which affects approximately 5-10% of the people during their life (Sabiha*et al.*, 2011).

Gastric hyperacidity and gastro-duodenal ulcer are very common global problem today. The pathophysiology of these disorders has focused on an imbalance between aggressive and protective factors in the stomach, such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors (Sakat*et al.*, 2012). The gram-negative spirochete, *Helicobacter pylori (H. pylori)*was first linked to gastritis in 1983. Since then, further studies of *H. pylori* has revealed that it is a major part of the triad, which includes acid and pepsin, that contributes to primary peptic ulcer disease. When *H. pylori* colonize the gastric mucosa, inflammation usually results. The causal association between *H. pylori* gastritis and duodenal ulceration is now well established in the adult and paediatric literature (Sung, 2001).

Another factor implicated in the pathogenesis of gastric ulcers is oxidative stress in the gastric mucosa. Studies have shown a positive correlation between increased generation of free radicals

and the extent of gastric ulceration in experimental animals (Hung, 2005). Agents that are currently available for the treatment of gastric ulcers act by either reducing gastric acid secretion (H_2-blockers, proton-pump inhibitors, anti-muscarinic agents), acting as physical barriers (sucralfate, colloidal bismuth sub-citrate), or increasing the mucous and bicarbonate secretion (prostaglandin analogues, carbenoxolone) (Hung, 2005).

Alcohol consumption is an independent risk factor for ulcers and increases the risk of ulcers in patients taking non-steroidal anti-inflammatory drug. Ethanol promotes oxidative stress, both by increased formation of reactive oxygen species (ROS) and by depletion of oxidative defences in the cell. The ROS-mediated induction of lipid peroxidation and protein oxidation are involved in the pathogenesis of ethanol-induced acute and chronic ulceration in the gastric mucosa, and scavenging free radicals is one of the mechanisms implicated in the healing of gastric ulcer (Hoek and Pastorino, 2002). Preventive antioxidants such as superoxide dismutase (SOD) and reduced glutathione peroxidase (GSH-Px) act as the first line of defence to prevent the destructive action of oxidative damage. The superoxide radical anion reacts with cellular lipids, leading to the formation of lipid peroxides that are metabolized to malonaldehyde (MDA) (Gao *et al.,* 2010). Moreover, by decreasing the secretion of bicarbonate (HCO_3) and mucus production, ethanol produce necrotic lesion in gastric mucosa. Ethanol has also been reported to activate tissue necrotic factor-α (NTF-α) and mitogen-activated protein kinases (MAPK) (Vidys*et al.,* 2010). It also initiates apoptosis, which leads to cell death. Ethanol after metabolism has been reported to release superoxide anion and hydroperoxy free radicals, which lead to an increased lipid peroxidation. Increase in lipid peroxide content and oxygen-derived free radicals results in a marked change in cellular levels and can cause membrane damage, cell death, exfoliation and epithelial erosion (Mahmood *et al*., 2011). Zinc is a vital component of numerous enzymes and is

a co-factor in signalling pathways involving zinc requiring proteins (Hershfinkel*et al*., 2007) as well as driving transcription factor activation and expression (Bao*et al.,* 2007). It is abundant in all cells of the human body (Tapiero and Tew, 2003), and plays a crucial role in numerous body functions. The role of zinc in immunity also involves T-cell independent aspects of the immune system, including natural killer (NK) as well as B-cells (Franker and King, 2004). Zinc is an essential trace element that is indispensable for its role in maintaining normal physiological function and cellular homeostasis (Little *et al*., 2010). Disturbances in zinc homeostasis have been observed in diabetes mellitus (Stadler*et al*., 2012) and several other pathologies, including cancer (Jayaraman, 2011), autoimmune disease (Delli*et al*., 2012), cardiovascular disease (Patrusher*et al*., 2012), and Alzheimer's disease (Lyubartseva*et al.,* 2010). Zinc has three major biological roles: catalytic, structural and regulatory (Chasapis*et al.,* 2011).

The catalytic and structural role of zinc is well recognized, and there are many noteworthy reviews on these functions (Maret*et al*., 2011). For example, zinc is a structural constituent in numerous proteins, including growth factors, cytokines, receptors, enzymes, and transcription factors belonging to cellular signalling pathways (Fukada*et al., 2*011). It is also implicated in numerous cellular processes as a co-factor for an estimated 3000 human proteins including enzymes, nuclear factors and hormones (Andreini*et al*., 2006).

Homeostatic mechanisms that modulate zinc absorption, distribution, cellular uptake, and excretion are vital for maintaining cellular function. Zinc fundamental and diverse roles in many cellular processes require that zinc delivery to tissues and cells, intracellular availability and intracellular distribution are tightly controlled. These processes are maintained through the

coordinated orchestration of a diverse family of transport proteins that modulate the uptake, efflux, and compartentalization of zinc (Mocchegiani*et al.*, 2008).

1.1Zinc Intake, Metabolism and Homeostasis

Animal products and seafoods constitute the richest sources of dietary zinc (Pa Tamba*et al.*, 2011), but are expensive and inaccessible for many of the world's poorest populations, making zinc deficiency a particularly widespread disorder in Asia and Africa. Dietary zinc is absorbed across the baso-lateral membrane of enterocytes in the duodenum and proximal jejunum, mediated by transporters including zinc transport protein-1(ZnTP-1), zinc passes through the portal circulation into the peripheral tissues via the liver (McMahon and Cousins, 1998). Over 60% of total body zinc is in skeletal muscle, with approximately 20% in bone marrow, liver, lungs and skin, being sites of rapid cell turnover, and it accounts for approximately 10% of the remaining body zinc. Hair also has a relatively high concentration of zinc, while the prostate gland and semen have particularly high concentrations. In humans, requirements for zinc change according to the individual's physiological state. Pregnancy, for example, sets an additional need equivalent to 5-7% of whole body zinc of a non-pregnant woman (Swanson and King, 1987), zinc in human is obtained only through the diet, and total body zinc status is influenced by: 1) rates of absorption; 2) excretion and losses; and 3) physiological and disease states. The balance in body zinc concentration is maintained by homeostatic mechanisms. Extracellular (plasma) zinc is labile and turns over rapidly in response to intracellular and tissue zinc utilization (Miller *et al*., 1994). Zinc absorption and excretion are driven by zinc transporters, with transmembrane domains, encoded by two solutelinked carrier gene families, Zip and ZnT (Liuzzi and cousins, 2004), which drive cellular zinc influx and efflux, respectively. Physiological responses, including the acute phase reaction to stressors such as infection, may modulate zinc turnover through zinc

binding or release by metalloproteins, the most potent of which are the metallothionin family (Brown, 1998). Seventy percent of extra-cellular zinc is transported around the body bound to albumin in the plasma; consequently, hypoalbuminaemia accompanying proteinenergy malnutrition and ageing lowers plasma zinc concentrations. However, only 0.1 % of body zinc is contained in plasma. Plasma zinc has a rapid turnover (150 times a day) to meet the needs of the tissues and is affected by recent dietary intake; therefore, plasma zinc is an unreliable measure of zinc status (King *et al*., 2000).

Although plasma zinc levels may be lowered in infection as part of the acute phase response, there are suggestions that this is true only in severe infection, perhaps when cytokines, the mediators of the acute phase response, reach a certain threshold, zinc level may also be lowered in adults, but not in children in whom severe infections may not be widespread (Pa Tamba*et al*., 2011).This suggestion is consistent with reports that although children with infections had lower mean plasma zinc concentrations, infection was not predictive of the proportion of children with lower than normal zinc status (Brown, 1993). Other studies showed no relationship between infection and plasma zinc concentrations (Frii*et al.,* 1996). In spite of these uncertainties and the known inadequacy of plasma zinc as a marker of zinc status, it remains the most widely used index.

However, novel approaches, including the use of metallothionein gene expression analysis, hold potential for true reflection of body zinc status (Aydemir*et al*., 2006).

1.2 Biochemistry and Mechanisms of Action

Zinc has anti-inflammatory action mediated through inhibition of nitric oxide formation (Abou-mohammad*et al*., 2000).There is a greater incidence of chronic diarrhea in zincdeficient children,

and zinc supplementation has historically improved chronic diarrhea in malnourished children (Bhutta*et al*., 2000) Zinc acts as an antioxidant through mechanisms outside its role in the antioxidant enzyme, superoxide dismutase. Zinc has been shown to protect vitamin E stores (Bunk *et al.,* 1998), stabilizes membrane structures, prevent Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) oxidation (Disilvestro and Blostein, 1997). It also protect against carbon tetrachloride-induced hepatitis, and maintains tissue levels of metallothionein, a potential free radical scavenger(Cousin, 1985) Recent studies exploring the mechanisms of zinc deficiency and immune suppression have shown zinc deficiency (like protein-calorie malnutrition) elevates the production of glucocorticoids through up-regulation of the hypothalamic-pituitary-adrenocortical axis (Fraker*al et.,* 2000). Emerging research in the mouse model has shown that zinc deficiency, through glucocorticoid release, actually causes a depletion of precursor T-and B-cells in the bone marrow and thymus, suppressing lymphocyte production. Zinc has a regulatory role in apoptosis, as it both induces and blocks cellular apoptosis, depending on the cellular concentration (Webb *et al* 1997). At very high concentrations, zinc can induce apoptosis in immune cells, possibly the mechanism responsible for the immune suppression of high supplemental doses of zinc. Apoptosis plays a role in many disease states, including autoimmune diseases, Alzheimer's and Parkinson's disease, stroke, ischaemia, cancer, and HIV infection (Webb *et al*., 1997).

1.3 Seeds of *Nigella sativa*

The seeds of black plant *(Nigella sativa*) belong to the botanical family of *Ranunculaceae* known throughout the world with different names such as Seed of blessing (*habbatulbaraka*), black cumin, black caraway, *Kalonji*, etc. The black seed oil is reported to be beneficial due to its

content of over a hundred components such as aromatic oils, trace elements and vitamins (Ali and Blunden, 2003). It is known to reduce the risk to diseases by strengthening immune system and protecting the body (Hajhashemi*et al.*, 2004; Tekeoglu*et al.*, 2006). Report however suggested that thymoquinone present in the oil might be the active component (Tekeoglu*et al*., 2006; Khattaband, 2007). The gastric protective effect of black seeds on ischaemic reperfusion injury (IRI) to gastric mucosa has also been reported (El-Abhar*et al.*, 2003, Kanter*et al*., 2004). In recent times, interest in medicinal properties of traditional plant-based therapeutics has increased worldwide resulting in a growing body of evidence showing therapeutic potential of traditional medicinal plants. *Nigella sativa* (black cumin or black seed) offers a good example. The name *N. sativa* comes from the Latin word, *Nigellus*, meaning black. *Nigella sativa* is an annual flowering plant that grows 20-30 cm tall and is native to Asia and the Middle East. The plant has small black seeds that have a slightly rough texture and an oily interior. They have a pungent bitter taste and a faint smell of straw- berries. The seeds have been used extensively in all levels and religions in Asia, the Middle East and Africa as herbal medicine, in religious ceremonies, and for cooking. In Islam, the seeds are regarded as a prophetic remedy with immense healing power. The prophet Muhammad once stated that the black seed can heal every disease, except death. Consequently, both Muslims and non-Muslims used it for thousands of years (Kanter*et al.*, 2004).

The seeds were also used by the ancient Romans in cooking and by Asian herbalists for many remedies, including migraines. The seeds have been found in several sites from ancient Egypt, including Tutankhamun's tomb. The seed and the oil are considered to be the greatest healing herb of our time in the East and thousands of scientific studies have been published on all aspects of medicine. *Nigella sativa* is used therapeutically to strengthen the

immune system, fight cancer, purify the blood and increase longevity. Since 1959, over 5000 studies at international universities and articles published in various journals have shown positive results, supporting its traditional uses (Cheikh-Rouhou*et al.,* 2007). Examples of plant medical uses mentioned in recent studies are: antiasthmatic (Kanter, 2009; Boskabady*et al*., 2010), antidermatophytic (Aljabre*et al., 2*005), antiepileptogenic (Ilhan*et al*., 2005), antioxidant (Zaoui*et al*., 2002; Mariod*et al*., 2009), antimetastatic (Al-JishiandAbuoHozaifa, 2003), immunomodulatory (Salem, 2005), blood glucose regulator (Meddah*et al*., 2009), leucocyte phagocytic regulator (Haq*et al.,* 1995), lipid peroxidation level regulator (Hosseinzadeh*et al*., 2007), therapeutic of liver damage (Mahmoud*et al.*,2002), neuropharmacological activity regulator (Al-Naggar*et al.,* 2003), therapeutic for pancreatic cancer (Banerjee *et al*., 2009).



Plate II. The flowering plant of *Nigella sativa (Ali,2003)*



Plate III: The seeds of *Nigella sativa*(Ali, 2003)

Moreover, while studies document the effectiveness of the black seed by itself, ongoing studies with the combination of other herbs have also documented healing potential. *N.*

sativa's chemical composition is diverse In addition to its primary ingredient, crystalline nigellone, the seeds contain 15 amino acids, complete proteins, carbohydrates, both fixed oils (84% fatty acids, including linolenic, and oleic), and volatile oils, alkaloids, saponin, and crude fibre, as well as minerals such as calcium, iron, sodium and potassium (Ghoshehet *al.*, 1999; Al-Batainaet *al*., 2003, Cheikh-Rouhouet *al.*, 2007). There are still many components in the plant seeds that have not been identified (Özcan and Akbulut, 2007).

1.3.1 Chemical composition of the seeds

Historically, the chemical investigations on the *Nigella sativa*seeds started in 1880, when Greenish published the first report concerning the presence of 37% oil and 4.1% ash (calcium salts) in the seeds. The percentage range of chemical composition of *N. sativa*seeds are; oil, 31-35.5%; proteins, 16-19.91%; carbohydrates, 33-34%; fibre, 4.5-65%;
Saponins, 0.013% and moisture, 5-7%.

1.4 Statement of Research Problem

Considering several side effects (arrhythmias, impotence and haematopoietic changes) of modern medicines such as H_2-blockers and proton pump inhibitors. Indigenous drugs possessing fewer side effectsshould be explored as a better alternative for the treatment of peptic ulcer. The prolonged use of synthetic anti-ulcer drugs leads to adverse drug reactions, and a search for new anti-ulcer agents that are available, affordable and retain therapeutic efficacy devoid of adverse drug reaction is desirable. A widespread search has been launched to identify new anti-ulcer therapies from natural sources; and*Nigella sativa* may be a potential source of agent for combating gastric ulcer.

1.5 Justification

Each year peptic ulcer disease (PUD) affects 4 million people around the world and it is the most common gastro-intestinal disorder in clinical practice (Zelickson*et al.,* 2011) Complications are encountered in 10-20% of the patients and 2-4% of the ulcer will perforate (Bertleff and Lange, 2010). Gastric hyperacidity and gastro-duodenal ulcer remains major health hazard in terms of both morbidity and mortality (Chaturvedi*et al.,* 2007).The natural history of peptic ulcer ranges from resolution without intervention to the development of complications with the potential for significant economic loss such as bleeding (Muzui*et al*.,2007).

1.6 Aim and Objectives

1.6.1 Aim

The aim of the study was to compare the gastric protective effects of *Nigella sativa* oil (NSO) and zinc gluconate on ethanol-induced gastric ulcer in Wistar rats

1.6.2 Objectives

They were to:

i. Compare the effects of zinc gluconate and *Nigella sativa* oil on gastric mucosal damage in rat
ii. Compare the effect of zinc gluconate and *Nigella sativa*oil on the volume of gastric secretion, titritable acidity and mean acid output in Wistar rats.
iii. Assess the extent of mucosal integrity of the experimental animals histologically.

1.7 Hypothesis

Nigella sativa oil and zinc gluconate have no significant effects on ethanol induced gastric mucosal damage and secretion in Wistar rats.

CHAPTER TWO

2.0 Literature Review

Peptic ulcer, characterized by mucosal damage is predominantly caused by *Helicobacter pylori*, and antiplatelet agents such as acetylsalicylic acid (Turkdogan*et al.,* 1999), (NSAIDs) such as oral bisphosphonates, potassium chloride and immunosuppressive medications (Elnagid*et al.,* 2008), serotonin reuptake inhibitors (Khan *et al.,* 2011), alcohol consumption, and cigarette smoking (Mekada*et al*., 2002). Anatomically, the ulcers mostly occur in the stomach and proximal duodenum (Bertleff and Lange, 2010). Globally, the incidence of peptic ulcer disease has fallen in recent years (Yuan and Hunt, 2006, Itasu*et al.,* 2011). In addition, cholinergic hypersensitivity and parasympathetic dominance, as well as gastric hypersecretion, play important roles in peptic ulcer disease. Zollinger-Ellison syndrome, antral G-cell hyperplasia, and bias in the antagonistic gastric hormones are correlated with gastric hypersecretion (Isenberg *et al.,* 1991).

Ulcer therapy has progressed from vagotomy to anticholinergic drugs, histamine H_2receptor antagonists, antacids and to proton-pump inhibitors (Wallace and Granger, 1996).In recent years, there has also been growing interest in alternative therapies and the use of natural products, especially those derived from plants (Rates, 2001). Plant extracts are some of the most attractive sources ofnew drugs and have been shown to produce promising results for the treatment of gastric ulcer (Alkofali and Atta,. 1999).

2.1Mechanisms of Gastric Mucosal Defence

2.1.1Mucus-bicarbonate-phospholipid barrier

The first line of gastric mucosal defence is represented by the mucus-bicarbonatephospholipid barrier. The surface of gastric mucosa is covered by a layer formed by mucus gel, bicarbonate anions and surfactant phospholipids (Lichtenberger 1999). The mucus layer is also able to prevent the penetration of pepsin, thus avoiding the proteolytic digestion of epithelium (Allen and Flemstrom, 2005). In addition, the luminal surface of mucus gel is covered by a film of surfactant phospholipids, which confers hydrophobic properties to the mucus layer (Lichtenberger, 1999). The mucus gel is secreted by surface epithelial cells along with low-molecular weight trefoil factor (CRF) family (TFF) peptides, which play a relevant role in the formation of the mucus layer (Newton *et al.,* 2000). The secretion of gastric mucus is regulated also by various gastro-intestinal hormones, including gastrin and secretin, as well as prostaglandins and acetylcholine (Allen and Flemstrom, 2005).The secretion of bicarbonate into the mucus gel layer is essential to maintain a pH gradient at the epithelial surface, which represents a first line of defence against gastric acid (Allen and Flemstrom, 2005).

2.1.2 Prostaglandins

Prostaglandins are 20 carbon fatty acids produced from arachidonic acid via enzyme cyclooxygenase (Kumar *et al.,* 2012).The gastric mucosa represents a source of continuous prostaglandin (PG) production, such as PGE_2 and PGI_2, which are regarded as crucial factors for the maintenance of mucosal integrity and protection against injuring factors (Halter *et al.,* 2001; Brzozowski*et al.,* 2005). It has been demonstrated that PG have the potential to stimulate almost all the mucosal defence mechanisms. In particular, they reduce acid output, stimulate mucus, bicarbonate and phospholipid production, increase mucosal blood flow, and

accelerate epithelial restitution and mucosal healing (Brzozowski*et al.*, 2005). Prostaglandins are also known to inhibit mast cell activation as well as leucocyte and platelet adhesion to the vascular endothelium (Halter *et al.*, 2001; Brzozowski*et al*., 2005).

2.1.3 Nitric oxide

Nitric oxide (NO) is synthesized from L-arginine via the catalytic action of a group of enzymes, the Nitric oxide synthase (NOS). NO has been studied to play an important role in the GI mucosal defence and the pathogenesis of mucosal injury (Kumar *et al.*, 2012). Suppression of NO synthesis renders the gastric mucosa more susceptible to injury. NO, inhibit recruitment of neutrophils to site of inflammation. NO reduces neutrophilic infiltration into the gatro-intestinal tract mucosa. The events related to the gastroprotective effects include a reduction in acid secretion and promotion of angiogenesis (Takeuchi *et al*., 2002).The gastro-protective effect of nitric oxide may be due to its rapid reactivity with various oxygen species in the biologic system. In addition, nitric oxide inhibit gastric secretion by suppression of histamine release from entero-chromaffin l- cells (Fretas et *al*., 2004).

2.1.4 Neuro-hormonal mechanism

Gastric mucosal defence is supported by mechanisms activated, at least in part, by the nervous system and hormonal factors. Experimental studies have demonstrated that central vagal activation stimulates mucus secretion and increases intracellular pH in the surface epithelial cells of the stomach (Laine*et al.*, 2008). Ghrelin, a hormone peptide produced by gastric A-like cells in rodents and PD1 cells in humans, is involved in the regulation of growth hormone secretion and appetite stimulation (Brzozowski*et al.*, 2005b). It also exert significant protective effects at gastric level, including the enhancement of mucosal blood flow via stimulation of NO and CGRP

release from sensory afferent nerves (Brzozowski*et al.,* 2005b). Glucocorticoids have been shown to support the mechanisms of protection at gastric level. These hormones are involved in the response to stress, and represent potent gastro-protective factors against injury (Filaretova*et al.*, 1998).

2.2 Mechanisms of Gastric Mucosal Damage

Gastric mucosal injury may occur as a consequence of various conditions, including alcohol intake, refluxed bile salts, stress, ageing and *H. pylori* infection, although the most important agents known to impair the mechanisms of gastric mucosal defence are represented by NSAIDs (Elnagid*et al.*, 2008).

2.2.1 Gastric acid secretion

The gastric acid is established as one of the major ulcerogenic factor for the induction of gastric ulcer disease. It has been reported that about 50% of gastric ulcer patients are pepsin and acid hypersecretors (Kumar *et al.*, 2012). On the other hand, gastric acid plays a stringent role in gastric defence. It is the first line of mucosal defence to prevent bacterial colonization and reduced their ability to enter the mucosal layer (Aihara*et al.*,2003). Acid secretion is suggested to be stimulated by three principal secretagogues histamine, acetylcholine and gastrin. The receptors on the surface of parietal cell includes H_{2}- receptors, responding to histamine released from the specialized mast cells, receptors that are sensitive to the muscarinic effect of acetylcholine released from vagus nerve, and probably receptors responsive to endogenous-circulating gastrin. Gastrin stimulates acid secretion either by direct stimulation of parietal cells or by release of histamine from entero-chromaffin cells (Lindstrom *et al*.,2001). The only source of the

acetylcholine (Ach) that can act directly on the parietal cell is postganglionic fibres of the enteric nervous system (Waldum*et al.,* 2002).

2.2.2 Composition of gastric juice

The fluid that is secreted into the stomach is the gastric juice. Secretion of gastric juice is regulated, through specific neural and hormonal pathways, by the act of eating and by the presence of food in the stomach and intestine. Three major constituents of gastric juice are the mucus component, enzyme and the aqueous component.

2.2.3 Mucus and bicarbonate

Mucus is a viscous, slippery gel that covers most of the mucosal surfaces throughout the gastro-intestinal tract (Neurtra and Forstner, 1987). The consistency and specialized properties of mucus are primarily due to its constituent gel-forming glycoprotein molecule known as mucins. Mucous neck cells within the gastric gland also secrete mucin, but its secretion is chemically distinct from that of the surface cells. The output or secretion of gastric mucins is under both local and neural control. As a defence against high acidity, the function of the adherent mucus is to provide a stable unstirred layer that prevents convenient mixing and rapid dissipation of the small amount of HCO_3^- secretion at mucosal surface (Flemstrom and Garner, 1989).

2.2.5 Pepsin

The principal enzyme of gastric juice is pepsin, although several other enzymes are present in smaller amounts. The less abundant enzymes include gastric lipase, which is maximally effective against triglycerides with short-chain fatty acids, and the proteolytic enzymes, cathepsin and

gelatinase, both of which, like pepsin, are from chief cells. Gastric pepsin is actually a heteregenous group of proteins, responsible for proteolytic activity of gastric juice (Samloff, 1989). These peptic proteins are secreted in the form of inactive precursor zymogen called pepsinogen.The pepsinogen can be broadly divided into two immunochemically distinct types, pepsinogen I (PG I) and pepsinogen II (PGII). In humans, PGI is synthesized, stored and secreted by chief cells and mucous neck cells only in the oxyntic region of the mucosa, whereas PGII is also secreted by mucous neck cells in the antral and pyloric mucosa. The heterogeneity of pepsinogen (and pepsin) arise from several factors, including variable number of pepsinogen genes, allelic variation within the genes and post-transitional modifications that may occur during processing. Independently of the heterogeneity, all the pepsinogens share the feature of conversion to enzymatically active- form, catalysed by the acidity of gastric juice: the lower the pH the more rapid the conversion, which is almost instantaneous below pH 2 (Taggart and Samloff, 1987).

2.2.6 Hydrochloric acid

In adult humans the stomach will typically secrete about 2-3L of gastric juice per day, and in some individuals up to 3-4 l/day is secreted. The acidity and ionic composition and gastric secretory products are not constant, but vary with the rate of volume flow or secretory rate. The concentration of H^+ and Na^+ show closest dependence on secretory rate: H^+ concentration asymptomatically approaches a maximum of about 150-160mm/l (pH-0.8) at high secretory rates, with a concomitant decline in Na^+ concentration of the juice. In addition, there are smaller increases in the concentration of Cl^- and K^+ as the flow increases (Neurtra and Forstner, 1987).

2.2.7 Intrinsic factor

Based on experimental observation and tests, Castle, (1975) proposed that absorption of vitamin B_{12} from the diet requires some factor that is secreted into gastric juice, known as intrinsic factor. In the absence of intrinsic factor, a severe, and potentially fatal, anaemia develops because of failure of the red blood cells to mature (pernicious anaemia). Intrinsic factor is a glycoprotein, which in humans is secreted by parietal cells along with HCl. The same secretagogues and intracellular messengers that stimulate HCl secretion also promote secretion of intrinsic factor (Donaldson, 1989).

2.3 Non-Steriodal Anti-Inflammatory Drugs and Peptic Ulcer

The ability NSAIDs to cause ulceration and bleeding in the upper gastro-intestinal tract was first documented by the endoscopic study of Douthwaite and Lintott in 1938.The pathophysiology of gastric injury associated with NSAID administration depends partly on cyclooxygenase inhibition and partly on cyclooxygenase-independent mechanisms, which result mainly from local direct actions (Scarpignato and Hunt, 2010).

Cyclooxygenase blockade has been shown to increase the susceptibility of gastric mucosa to NSAID-induced injury by suppression of a number of prostaglandin-mediated protective functions. Prostaglandins reduce the activation of neutrophils and the local release of reactive oxygen species (ROS). The production of prostacyclin by the endothelium of mucosal microcirculation is also highly relevant in ensuring a tonic inhibition of neutrophil adhesion. Therefore, NSAIDs can shift the mucosal balance towards the recruitment and endothelial adhesion of circulating neutrophil through the inhibition of prostaglandin biosynthesis (Whittle, 2002). Once adhered, neutrophils clog the microvasculature, causing a local decrease in mucosal blood flow and a marked release of tissue damaging factors, including proteolytic enzymes and

leucotrienes. These enhanced the vascular tone; exacerbate tissue ischaemia, andstimulate the production of ROS. These promote the destruction of intestinal matrix, leading to a severe degree of local tissue necrosis, particularly in the presence of a low luminal pH (Whittle, 2002; Jimenez *et al.*, 2004).

2.4 *Helicobacter pylori* and peptic ulcer disease

Helicobacter pylori are corkscrew-shaped bacteria that commonly live and multiply within the mucous layer that covers and protects tissue lining the stomach and duodenum. The bacteria penetrate and disrupt the mucous layer and inflame the stomach lining, leading to chronic inflammation or gastritis. *H. pylori* infection impairs negative feedback of acid secretion by disrupting somatostatin release and its subsequent inhibitory control of gastrin release from G cells, leading to functional impairment of antral-fundic neural connections (Moses *et al*., 1992). The immuno-pathogenic aspect of ulcerogenesisis primarily driven by the influx of neutrophils and macrophages into the gastric mucosa in response to *H. pylori* colonisation, with the release of lysosomal enzymes, leukotrienes and ROS that impair mucosal defence (Craig *et al.,* 1992). *H. pylori* virulence factors, including adaptive enzymes, toxins and mediators of inflammation, lead to bacterial persistence in the stomach and disruption of the gastric mucosal barrier. Production of alkaline ammonia by *H. pylori* urease prevents D cells in the antral glands from sensing the true level of acidity, leading to inappropriate release of somatostatin. It has been suggested that *H. pylori* strains from ulcer patients produce higher amounts of this enzyme than those people without ulcers (Malfertheiner, 2009). A wide range of *H. pylori* virulence factors has been associated with more serious disease outcome. Of these, the cag pathogenicity island (cagPAI), the vacuolatingcytotoxin A (VacA) and the duodenal ulcer promoting gene A (dupA) have received the most scrutiny in relation to PUD (Backer *et al.,* 2010)

2.5 Other Risk Factors

Other causes of gastric ulcers include conditions that can result in direct damage to the wall of the stomach lining such as alcoholism, radiation therapy, burns, and physical trauma to the abdomen. In addition to NSAIDs, over-use or misuse of virtually any oral pharmaceuticals can also precipitate gastric inflammation and ulceration, especially those medications used to treat osteoporosis called bisphosphonates (Actonel, Fosamax and others) (Malfertheiner*et al*., 2009) .Risk factors that have been identified for gastric ulcers include tobacco smoking and chewing, moderate-to-high levels of alcohol consumption, moderate-to-high levels of coffee consumption, high-stress occupations, severe emotional trauma, long-term use of pharmaceutical medications, chronic pain, surgical procedures, advancing age and heredity (Salih*et al*., 2007).

2.6 Signs and Symptoms of Gastric ulcer

Burning-type abdominal pain is the most common symptom of gastric ulcers. With gastric ulcers, the pain is felt predominantly in the middle of the abdomen, although it can be felt from the navel to the sternum and clavicles, and posteriorly in the thoracic region of the spine (Malfertheiner*et al.*, 2009). The pain is caused by the tissue ulceration, which exposes nerve fibres, aggravated by gastric acid coming in contact with the ulcerated area. The pain is typically worse with an empty stomach (about three hours after meals), although it may flare-up after eating spicy and acidic foods. Conversely, the burning pain may be temporarily relieved by eating certain foods or drinking beverages that buffers stomach acid, such as milk. The pain from gastric ulcers is rarely constant; rather, it fluctuates during the day, often flares up during the night, and might disappear for many days only to return again (Malfertheiner*et al.*, 2009).

Gastric ulcers often bleed and produce distinctive signs and symptoms. Signs of bleeding in the upper digestive tract, including the stomach, are different from the signs from bleeding from the large intestine and bowel. Bleeding from gastric ulcers usually lead to black or tarry stool and vomit that looks like coffee grounds (if the blood is old and partially digested) or coloured bright red (if the blood is fresh) (Fauci*et al.,* 2008).Tarry stool from internal bleeding, known as malena, is especially foul-smelling due to the oxidized iron of the haemoglobin. In contrast, bleeding from ulcer in the gastro-intestinal tract usually produces bright red blood in the faeces, but no blood in the vomitus. In fact, vomiting is not a typical symptom of gastro-intestinal bleeding. In addition to intense pain and vomiting, other signs and symptoms of gastric ulcers include loss of appetite, unexplained weight loss, heartburn, indigestion, belching, bloating and nausea. If left untreated, peptic ulcers can result in anaemia from lossof blood, infection (peritonitis) from tissue perforation, and scar tissue that can block passage of food through the digestive tract. Sudden, excessive bleeding can be life threatening, especially if the ulcer erodes one of the blood vessels, such as the gastroduodenal artery (Cullen *et al*., 1997). Stomach cancer is between three and six times more likely to develop from gastric ulceration caused by *H. pylori* infection (Marfertheiner*et al.,* 2011). On the other hand, about four percent of stomach ulcers are actually caused by a malignant tumour, so sometimes stomach biopsies are needed to rule-out cancer (Fauci*et al*., 2008)

2.7 Treatment of Peptic Ulcer

The modern history of peptic ulcer disease has followed a remarkable course and lead to widespread acceptance of the notion that the majority of gastric ulcers are caused by *H. Pylori.* The institution of treatment protocols such as ―triple therapy, which has had dramatic effects, which produces greater than 90 percent initial eradication rate, and less than 10 percent

re-infection rate at five years (Qasim, 2002). Triple therapy is the use of a proton-pump inhibitor or H_2-blocker (which both reduce gastric acid secretion), with either two different antibiotics or an antibiotic combined with bismuth salicylate (found in Pepto-Bismol). The discovery of effective and potent acid suppressants and the identification of the largely bacteria cause of peptic ulceration are the two most important developments that are associated with the overall decreased rates seen with peptic ulcer disease. Additionally, the morbidity associated with *H. pylori* infection has dramatically decreased from 1993 to present date mainly due to triple therapy (Bashinskaya*et al.,* 2011). Although the overall rates of peptic ulcer disease have decreased, incidence of gastric ulceration has increased over the last few decades due to increased NSAID use.

Thus, antibiotics or triple therapy are not given to patients with gastric ulcers caused by NSAIDs; rather, their pain medications are either reduced, eliminated or altered, and acidreducing medications may be recommended for a few months to allow the ulceration to heal (Qasim, 2002).

2.7.1 Proton-pump inhibitors

Proton-pump inhibitors are a group of drugs that display pronounced and long-lasting reduction of stomach acid production. They are among the most widely-sold drugs in the world and are generally considered the most potent inhibitors of acid secretion, more so than an earlier class of inhibitors called H_2-receptor antagonists (or simply H_2-blockers) (CPA, 2008) Proton-pump inhibitors act by irreversibly blocking the hydrogen/potassium adenosine triphosphatase enzyme system (commonly called the gastric proton pump) of the stomach's parietal cells. The proton-pump is the final stage in stomach acid secretion, being directly responsible for secreting hydrogen ions into the gastric lumen. Protonpump inhibitors reduce gastric acid secretion by up

to 99 percent. They are given in an inactive form, which readily cross-cell membranes and enter into parietal cells, where they become activated by the acidic environment (Sachs *et al*, 2006).

2.7.2 H_2-blockers

H_2 blockers, also known as histamine blockers or H_2-receptor antagonists, are competitive antagonists of histamine at the parietal cell H_2-receptors in the stomach. H_2-blockers suppress the secretion of hydrochloric acid by parietal cells via histamine released by entero-chromaffin-like cells in the stomach. It is blocked from binding on parietal cell H_2receptors, which stimulate acid secretion. Other substances that promote acid secretion (such as gastrin and acetylcholine) have a reduced effect on parietal cells when the H_2receptors are blocked. H_2-blokers are still commonly used for the treatment of dyspepsia, but they have been surpassed in popularity by the more effective proton-pump inhibitors for the treatment of gastric ulcers (Marshall and Warren, 1984).

2.7.3 Antibiotics

Antibiotics are usually prescribed for only two weeks at a time in order to avoid side effects. Relatively common side-effects of antibiotic use include acquired resistance to antibiotic therapy, serious allergic reactions (including anaphylaxis), nausea, stomach upset, diarrhea, insensitivity, disruption of the intestinal flora and fauna, systemic overgrowth of pathogenic bacteria (such as *Clostridium difficile*) and yeast species (such as *Candida albicans*), and numerous potential interactions with other drugs (PDR, 2009) In the U.S., clarithromycin-based triple therapy (combined with a proton-pump inhibitor and either amoxicillin or metronidazole for 10-14 days) is considered the standard treatment for an ulcer caused by *H. pylori*; however, other protocols are also popular. Research shows higher cure rates with 14 days of treatment, although side effects become much more probable and severe beyond this time frame (CPA 2008). At least four

weeks after the initiation of antibiotic treatment, the patient is tested by the doctor using a breath or stool test to be sure the *H. pylori* infection has been eradicated. Blood tests are not useful after treatment because a patient's blood can test positive for *H. pylori* even after the bacteria have been eliminated. If the infection remains, another two-week round of triple therapy is usually recommended, but typically with a different combination of antibiotics to prevent resistance (although *H. pylori* resistance to amoxicillin is considered rare). Another strategy, called ―salvage‖ or quadruple therapy, involves adding bismuth salicylate compounds to the treatment protocol (PDR, 2009).

2.7.4 Bismuth salicylate

Medications that protect the lining of the stomach and small intestine from the effects of acid are calledcytoprotective agents and include the prescription medications sucralfate(Carafate) and misoprostol (Cytotec), and over-the-counter agents such as bismuth salicylate (Pepto-Bismol). Bismuth compounds may also directly kill *H. pylori*, although their antibacterial actions should not be viewed as a replacement for conventional antibiotics. Bismuth salicylate, like all salicylates (especially aspirin), can cause serious bleeding problems, when used alone in patients with bleeding ulcers. Bismuth quadruple therapy usually involves combining bismuth salicylate with a proton-pump inhibitor, tetracycline and metronidazole for 10-14 days (PDR, 2009). The main reasons for adding bismuth salicylate to a treatment regime for gastric ulcers is, if the patient is still infected with *H. pylori* after a trial of triple therapy, or if the patient cannot take amoxicillin (a penicillin-like antibiotic) because of a penicillin allergy, or if the patient has been treated before with a macrolide antibiotic, such as clarithromycin. Side effects from bismuth salicylate are considered rare, but the most common are benign and include darkening of the stools and/or tongue, and a metallic taste in the mouth (PDR, 2009).

2.8 Biological effects of Zinc Gluconate

2.8.1 Effect of zinc on gastric mucosal injury.

Zinc and its compounds have demonstrated gastro-protective effects against various ulcerogenic agents (Rainsfold*et al.,* 1992). Specifically, pre-treatment with zinc monoglycerolate and zinc acexamate significantly decreased NSAID-induced mucosal injury (de-Rodriguez *et al.,* 1994). Proposed cytoprotective mechanism includes membrane stabilization, improved gastric mucosal blood flow, improved prostaglandins synthesis and increased mucus secretion (Vaananem*et al*., 1991). Alternatively, the protective effect of zinc could be mediated through the activation of, and/or generation of sulphydryl compound as has been reported in previous studies (Mann *et al*., 1992).

2.8.2 Effect of zinc on immune system

Zinc affects multiple aspects of the immune system (Prasad, 2008). Zinc is essential for normal development and function of cell-mediating innate immunity, neutrophils, and natural killer cells. Macrophages are also affectedby zinc deficiency. Phagocytosis, intracellular killing a cytokines production are all affected by zinc deficiency.The growth and function of T-and B-cells are also affected adversely due to zinc deficiency. Zinc is needed for DNA synthesis, RNA transcription, cell division and cell activation. Apoptosis (programmed cell death) is potentiated by zinc deficiency. Zinc deficiency adversely affects the secretion and functions of cytokines, the basic messengers of the immune system. Zinc functions as an antioxidant and stabilizes membranes. It deficiency leads to atrophy of the thymus and lymphoid tissue in experimental animals (Ananda, 2009). In young adults, zinc deficient mice, thymic atrophy, decrease in absolute number of splenocytes, and decreased responses to both T-cell-dependent and T-cell-independent antigens

have been observed (Prasad, 2008). Animals maintained on a zinc-deficient diet for as little as two weeks developed a severe impairment in their ability to generate a cytotoxic T-killer response to the tumour challenges (Frost *et al*, 1981).

2.8.3 Effect of zinc on oxidative stress

Studies in humans (Leone *et al*., 2006), animal models (Oteiza*et al*., 2000), and cultured cells, provide support for the link between Zn deficiency and its regulation of oxidative stress. Zinc is an essential co-factor for the activity of a number of proteins that play a role in combating oxidative stress, such as metallothionins and Cu/Zn SOD (Dreosti, 2001). Zn is also a cofactor for proteins involved in mediating DNA damage response and repair, such as the p53 tumour suppressor protein (Theocharis*et al.*, 2004). Therefore, suboptimal Zn intake may promote the generation of oxidative stress, and single and double DNA strand breaks similar to radiation-induced DNA damage (Ames *et al*., 2000). Compromised DNA integrity, impaired functioning of DNA repair enzymes and loss of DNA surveillance mechanisms are associated with increased risk of cancer initiation and progression (Song *et al*., 2009, Sharif *et al.*, 2011).

2.9 Biological Effect of *Nigella sativa*

Nigella sativa oil (NSO) as well as thymoquinone (TQ) have been shown to prevent oxidative injuries. Incorporation of ethanolic extract of NS seeds in corn oil retarded the oxidative deterioration of triglycerides (Bassim-Atta *et al.*, 1998). Oral feeding of *N satva* oil or TQ has protected the methionine-inducedhyperhomocysteinaemia by enhancing the antioxidant status and amelioration of triglycerides, lipid peroxidation and cholesterol concentration in plasma (El-Saleh *et al*., 2004). Intraperitoneal (i.p) injection of NSO and TQ have also shown protective effects on lipid peroxidation process during ischaemiareperfusion injury (IRI) in rat hippocampus

(Hosseinzadeha, *et al.*, 2007), The attenuation of oxidative stress by radical scavenging properties of NSO has been clearly indicated in pentylenetatrazol (PTZ) induced seizure-kindled mice (IIhan*et al*., 2005). TQ is a potent chemo-preventive agent and has been shown to suppress the Fe-NTA-induced oxidative stress, hyperproliferative response and renal carcinogenesis in Wistar rats (Khan, *et al*., 2005). Oral feeding of the diet containing NS powder at 10% level antagonized the oxidative stress effects induced by hepatocarcinogens such as dibutylamine and $NaNO_3$ in Swiss albino rats by normalizing GSH and NO levels (Gendy*et al.,* 2007). Dietary supplementation of seed powder inhibits the oxidative stress, caused by oxidized corn oil in rats (Al-Othman *et el*., 2006). Treatment with NS for 6 weeks in broiler chicks prevented the liver from oxidative stress by decreasing hepatic lipid peroxidation and increasing the activities of enzymes such as catalase, glutathione-S-transferase, adenosine deaminase and myeloperoxidase (Sogut*et al*., 2008). Most recently, the antioxidant activities of crude methanolic extract of black cumin seed cake were also investigated and some of the phenolic compounds such as hydroxybenzoic, syringic and p-cumaric acids were identified with significant antioxidant properties under in vitro systems (Mariod*et al*., 2009)

2.9.1 Gastro-protective activity of *Nigella sativa*

Acute gastric ulcer is mainly caused by ROS and lipid peroxidation. Therefore, treatment with antioxidant and free radical scavenger can reduce gastric mucosal damage. *N.Sativa* prevents alcohol induced increase in thiabarbituric acid reactive substances (TBARS), an index of lipid peroxidation, and reduced gastric glutathione (GSH) content, enzyme activities of gastric superoxide dismutase (SOD), Glutathione-S-transferase (GST).TQ protected against the ulcerating effect of alcohol and mitigated most of the biochemical adverse effects induced by alcohol in gastric mucosa, but to a lesser extent than *N. Sativa* .Neither *N. Sativa* nor TQ affected

catalase activity in gastric tissue and both NSO and TQ possess gastro-protective effect against gastric lesion, which may be related to the conversion of the gastric mucosal redox state (Arslan*et al*., 2005)). Alcoholic extract of *N.Sativa*significantly decreased the volume of gastric acid secretion, free acidity, total acidity and ulcer index with respect to control in pyloric ligation and aspirin induced model system of gastric ulcer (Andrews *et al.*, 2000). Indomethacin induced changes of gastric juice volume, pH, acid-output and ulcer index were attenuated by ethanolic extract of *N. Sativa* in Wistar rats. The observed effect was attributed to anti-inflammatory and/or antioxidant activity of the extract (Arslan*et al*., 2005).

2.9.2 The effects of thymoquinone

Thymoquinone (TQ) was considered as one of the major components of *Nigella sativa*volatile oil. For this reason, the influence of this substance (whether natural or synthetic) was explored in most of the systems investigated for the volatile oil (Arslan*et al.*, 2005).

CHAPTER THREE

3.0 Materials and Methods

3.1 Plant Material

The *Nigella sativa*(black seed) seeds were purchased on 8thMarch 2013 at Sabon-Gari market in Zaria. The seeds were identified and authenticated at herbarium section of the Department of Biological Sciences of Ahmadu Bello University, Zaria, by Mal. U.S Gallah with a voucher No.01713 and part was kept for future reference. The extraction and preliminary phytochemical screening of the oil from the seeds were carried out employing the standard screening test (Evans and Trease, 1996) at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, using cold press method (Fils, 2000). The cold press method is a method that does not require heat, which means the oil, retains all its natural nutrients.

3.2 Animals and Management

A total of 50 male rats weighing between 150 - 200g were purchased from the animal house of Department of Human Physiology, Ahmadu Bello University, Zaria. The animals were kept in separate cages with wide meshed raised bottom to prevent *coprophagia*. The animals were housed under normal environmental temperature with a standard pellet diet and water given *ad libitum*. The animals were allowed to adjust to the laboratory environment for two weeks. They were divided into five groups of 5 rats each for gastric mucosal damage and gastric secretion

studies respectively. They were administered with the following; 10ml/kg of distilled water, 50 mg/kg of zinc gluconate, 5 ml/kg of *Nigella sativa oil* and 50mg/kg of zinc gluconate plus 5ml /kg NSO orally for 7 days.

3.3 Induction of Gastric Ulcer

The gastric ulcer was induced according to Shay *et al*., (1954). The animal to be operated upon were kept in separate cages, with raised wide meshed wire bottom to avoid *coprophagia* (Basso *et al.*, 1983) for 48 hours before the experiment to ensure complete emptying of the stomach. The rats were deprived of food but water was provided *ad libitum*. One hour before the experiment the water was removed from the cages and the rats weighed. Calculated doses of zinc gluconate, *Nigella sativa* oil (NSO), combination of zinc gluconate and *Nigella sativa* oil respectively were administered orally. After thirty minutes later, the rats were administered absolute ethanol orally at a dose of 1ml/kg, except the control group, and were allowed to recover for one hour before they were sacrificed by cervical dislocation (Harbi*et al*., 1997).

3.3.1 Quantitative assessment of mucosal damage

The stomach was removed and opened along the greater curvature, rinsed slowly under a running water, and stretches out as much as possible on Whitman's filter paper mounted on a ceiling board. The ulcerated stomach of each animal was measured with a transparent millimetre scale ruler and result for each group were expressed as ulcer index (UI) in millimetre of mean ulcer± SE (standard error).. Preventive index percentage (P.I %) was calculated according to method of Hano*et al.*, (1976) as follows;

$$.P.I = \frac{\text{UI Ethanol - UI of (Zn or NSO) + UI Ethanol}}{\text{UI Ethanol}} X\ 100$$

.

After enumeration, the stomachs were fixed in 10% formal saline solution for histopathological examination

3.4 Gastric Secretion Studies

3.4.1 Collection of gastric secretion

The animals were deprived food for 48 hours with access to water *adlibitum* for gastric emptying and a steady state of gastric secretion were kept in separate cages. Under light anesthesia using chloroform the abdomen was shaved, and midline incision was made extending 2cm from the xiphoid process posterioly. The duodenum was identified; the junction between the pylorus bulb and duodenum was picked gently. Pylorus ligature was made with a thread carefully, to avoid damage to blood vessels. The abdomen was closed with an uninterrupted suture and was thoroughly washed with normal saline (Shay *et al.,* 1954). The animals were allowed to recover for 3 hours after which were killed by decapitation (Satoh *et al.,* 1983).The abdomen of the animals were opened, the esophagus was ligated, the stomach was removed and washed with normal saline. An opening was made along the greater curvature to collect the gastric contents, into graduated centrifuge tube and centrifuged at 3000 revolution per minute for 10 minutes for estimation of gastric secretion.

3.4.2 Analysis of Gastric Juice

3.4.3 Determination of the volume of each sample after centrifugation

The volume of each of the gastric content was determined after the pyloric ligation

3.4.4 Determination of titritable acidity:

For each stomach, 0.2 ml of gastric juice was titrated against 0.01N NaOH using an end point of pH, 7.0 as determined with phenol red as indicator (Grossman 1963). It was calculated as the milliequivalents per litre (mEq/L), equal to the amount of 0.01 N Na0H in ml required to neutralize 0.2 ml of the gastric juice (Davenport, 1977).

$$\frac{\text{Volume of 0.01N NaOH (ml) which neutralizes 1ml of gastric juice}}{10} \times 100$$

3.4.5 Determination of total mean acid output

The total mean acid output was calculated as micro equivalents per hour (μEq/h) by multiplying the volume of gastric juice in ml/h by titritable acidity in (mEq/l) (Brodie 1971)

3.5 Histopathological studies

Specimens of the gastric tissues of each rat were fixed in 10% buffered formalin for histological evaluation at Ahmadu Bello University Teaching Hospital, Zaria, (Abdulla *et al*., 2010; Ketuly*et al*., 2011).

a. Tissue fixation: Formal saline 10% was used to fix the gastric tissue in cold container.
b. Dehydration: The fixed gastric tissues were dehydrated to remove water from the tissue using a series of graded alcohol (70, 80, 90, 100%).
c. Clearing: Thedehydrants removed/cleared using xylene or toluene.
d. Embedding: Molten embedding agent (paraffin) was introduced into the tissue to enable the tissue to be cut into sections.
e. Sectioning: The embedded tissues were cut into sections with a microtome (a knife). Section cut floated on a warm water bath that helps remove wrinkles. They were picked

up on a glass microscopic slide. The glass slides were placed in a warm oven for 15 minutes to help adhere to the section to the slide.

f. Staining: The slides were deparaffinized by running them through xylene to alcohol and water before staining is done to allow water soluble dye to penetrate the sections.

Hematoxyline and Eosin (HαE) were used for staining various cellular inclusion of the liver and kidney. The tissue were stained by modified aldehyde fuctisin stain technique.

g. Mounting/viewing: The stained section on the slide were covered with thin glass to protect the tissue from being scratched in order to provide better optical quality were viewed under a photo microscopic model (400) view with a magnification of (X 250). Photomicrographs of the prepared slides were taken from Ahmadu Bello University Teaching Hospital, Zaria.

3.6 Statistical analysis

This was expressed as mean ± SEM. The data was analysed by One-way ANOVA, followed by Turkey's posthoc test. Results were considered statistically significant when P values were less than 0.05 ($p \leq 0.05$)

CHAPTER FOUR

4.0 Results

4.1 Ethanol-Induced Gastric Mucosal Damage

Group I are the control group that received 10ml of distilled water orally, showed a normal architecture of the gastric mucosa with ulcer index of 0.0 ± 0.0 and preventive index of 100% as presented in table 4.1. Group II were administered 1ml of absolute ethanol that produced 100% gastric ulcer, with an index of 10.3 ± 0.9 mm. Group III were administered 1ml of absolute ethanol with 5ml/kg of *N. sativa* oil orally. The *N. sativa oil* treated group produced a significant decreased in the ulcer index of 5.5 ±1.0 mm and a preventive index of 48.8% compared to the control group. Group IV were treated with 1ml of absolute ethanol and 50mg/kg of zinc gluconate orally. The administration of zinc gluconate more significantly reduced the ulcer index to 4.8 ± 0.3 mm with a preventive index of 53.6% as compared to control group. While Group V were treated with 1ml of absolute ethanol plus 5ml/kg *N. Sativa oil* and 50mg/kg of zinc gluconate orally. The group further produced a significant reduction in the ulcer index to 3.8 ± 1.4 mm, with a prevent index of 63.4% as compared to the control group.

4.2 Gastric secretion studies in rats

Values of different gastric secretion parameters in rats are presented in Table 4.2. Group I are control that were administered 10ml/kg of distilled water orally to produced 0.72 ± 0.08 ml/3h of gastric juice, 36.00±4.30 mEq/L of titratable acidity and a mean acid output of 27.46±6.52 µEq/h. Group II received 1ml of absolute ethanol orally showed a significant increase in the volume of 1.10 ± 0.51ml/3h of gastric juice, titratable acidity of

Table 4.1 Ulcer and preventive indices of ethanol-induced gastric mucosal damage in Wistar rats

Group	Treatment	Ulcer Index (mm) Mean ± SEM	Preventive Index (%)
I	Control	0.0 ± 0.0	100.00
II	Ethanol	10.3 ± 0.9	0.00
III	NSO	5.5 ± 1.0*	48.80
IV	Zn. gluc.	4.8 ± 0.3*	53.60
V	NSO + Zn	3.8 ± 1.4*	63.40

* Denotes a statistically significant difference when compared to control at $p \leq 0.05$

55.00 ± 19.62 mEq/L and mean acid output of 91.30 ± 62.31µEq/h as compared to control

Group III were treated with 1 ml of absolute ethanol and 5ml/kg of *N. Sativa oil* orally showed a

decrease in volume of 0.50 ± 0.14 ml/3h of gastric juice, titritable acidity of 25.00±6.89 mEq/L and mean acid output of 16.30 ± 7.34 µEq/h as compared to control group.With regard to Group IV the animals in this group were treated with 1ml of absolute ethanol plus 50mg/kg of zinc gluuconate, produced a slight increase in the volume 0.82 ± 0.15 ml/3h of gastric juice, with a corresponding increase in titratable acidity of 41.00 ±7.65 mEq/L and mean acid output of 38.30 ±15.25µEq/h as compared to the control group. Group V were treated with 1ml of ethanol plus 50mg/kg of zinc gluconate and 5ml/kg of *N. Sativa oil* showed a significant reduction in the volume of 0.50±0.14ml/3h of gastric juice, with titratable acidity of 25.00 ± 6.89mEq/L and mean acid output of 16.30 ± 7.34 µEq/h as compared to control.

Table 4.2 The Volume of Gastric Juice, titritable acidity and mean acid output in rats.

Group	Treatment	Volume of gastric juice (ml/3h)	Titratable acidity (mEq/L)	Mean acid output (µEq/h)

I	Control	0.72 ± 0.08	36.00 ± 4.30	27.40 ± 6.52
II	Ethanol	1.10 ± 0.39	55.00 ± 19.62	91.30 ± 62.31
III	NSO	0.56 ± 0.17	28.00 ± 8.68	21.50 ± 12.85
IV	Zinc gluc	0.82 ± 0.15	41.00 ± 7.65	38.30 ± 15.25
V	NSO+ Zinc glu	0.50 ± 0.10	25.00 ± 6.89	16.30 ± 7.34

The mean difference is not statistically significant when compared to control $p > 0.05$

4.3 Histopathological Findings

The group 1 animals were the control group treated with distilled water orally revealed a normal structure of the gastric cells (Plate 1). Group II animals were ethanol group showed a disruption

in gastric mucosal integrity and enlargement of gastric pits and infiltration by inflammatory cells (Plate 2) as compared to the control group. Group III were treated with ethanol and *N.sativa oil* showed the size of the gastric mucosa was preserved (Plate 3). Furthermore, Group IV are ethanol and zinc gluconate treated group showed a preserved outline of gastric mucosa, however, some areas of focal necrosis were noticed (Plate 4). The group V were treated with *N. sativa* oil plus zinc gluconate showed general outline of the mucosa and the size of the mucosa appeared preserved (Plate 5).

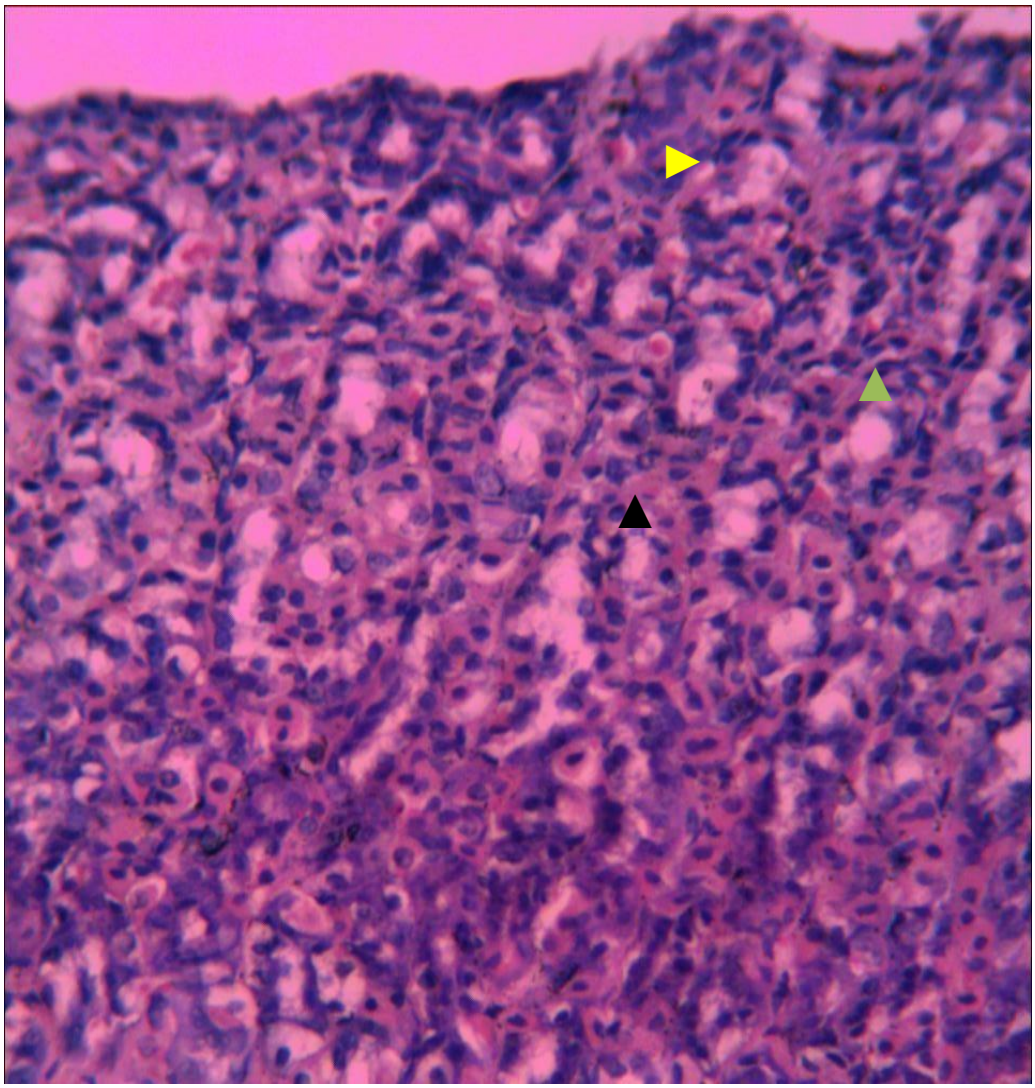

Plate 1: Photomicrograph of a section of gastric tissue from the control group (x250; H&E stain) showing normal structure of the gastric cells. Surface mucous secreting cells (yellowpointer), parietal cells (green pointer) and zymogenic/chief cells (black pointer).

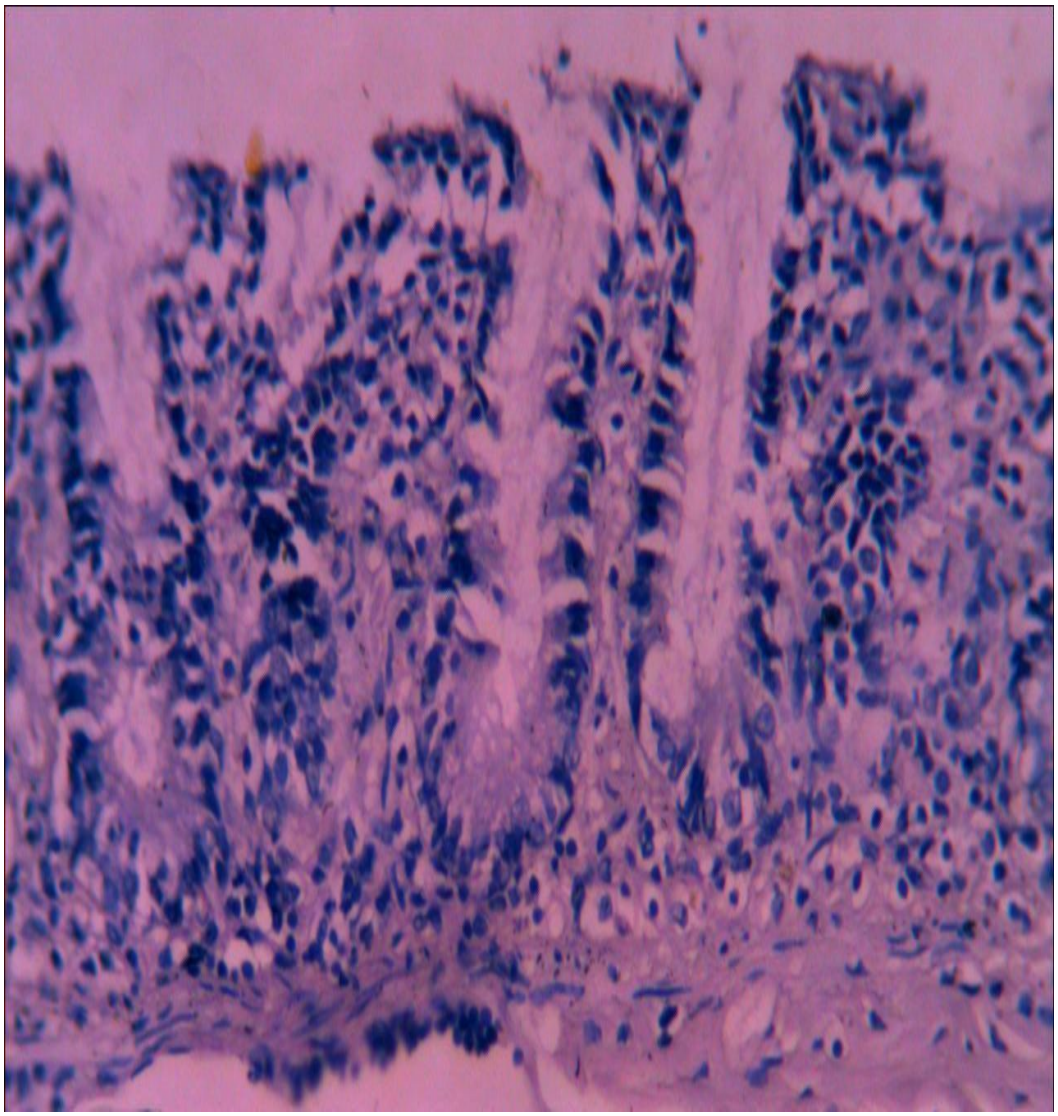

Plate 2: Photomicrograph of a section of gastric mucosa from Ethanol treated Group (x250; H&E) showing necrotic cells (pyknotic cell (arrows), karyohexic cell (green arrow) and necrotic surface mucous secreting cells (purple arrow). There is also enlargement of the gastric pits (GP) and infiltration by inflammatory cells (black arrow).

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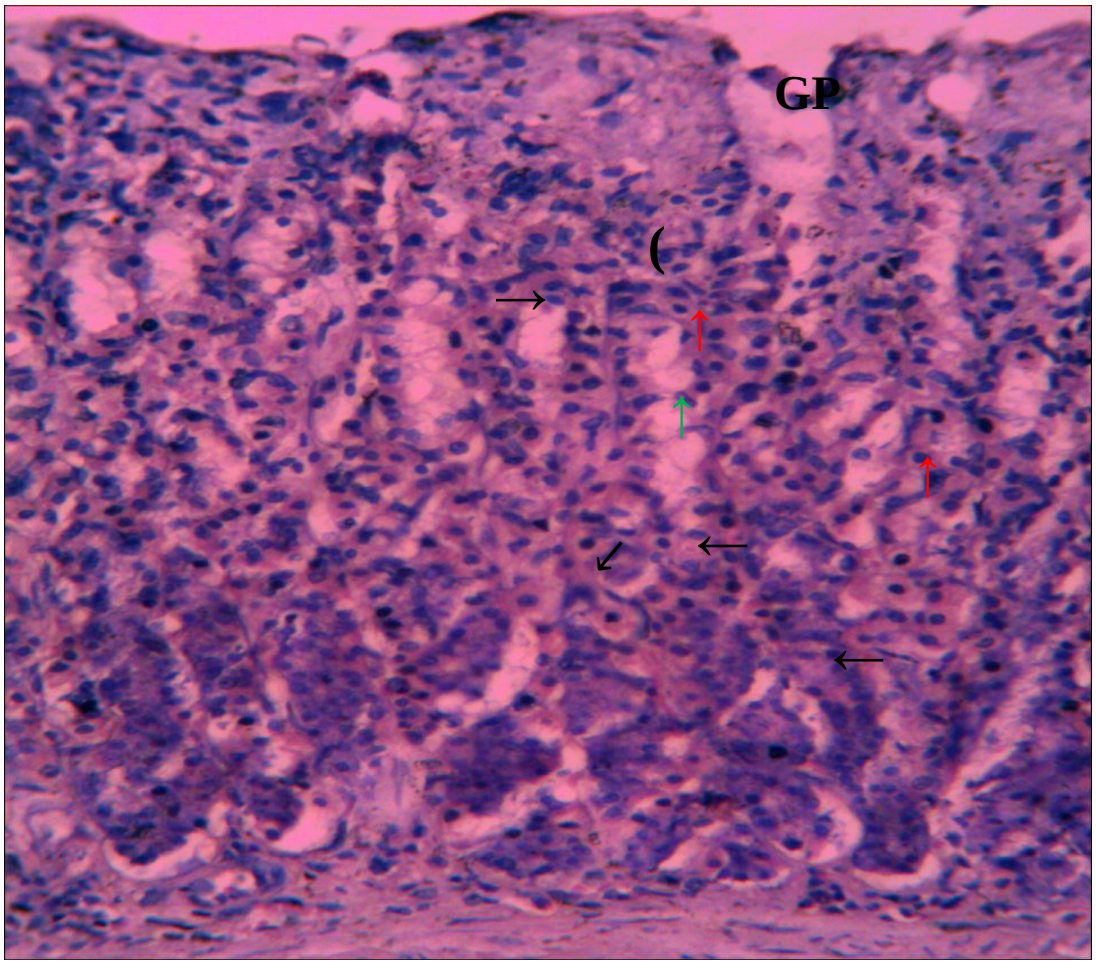

Plate 3 Photomicrograph of a section of gastric mucosa from NSO treated Group (x250; H&E) showing areas of focal necrosis (black arrows) characterized by darkening of nuclei of cells. (GP= gastric pits)

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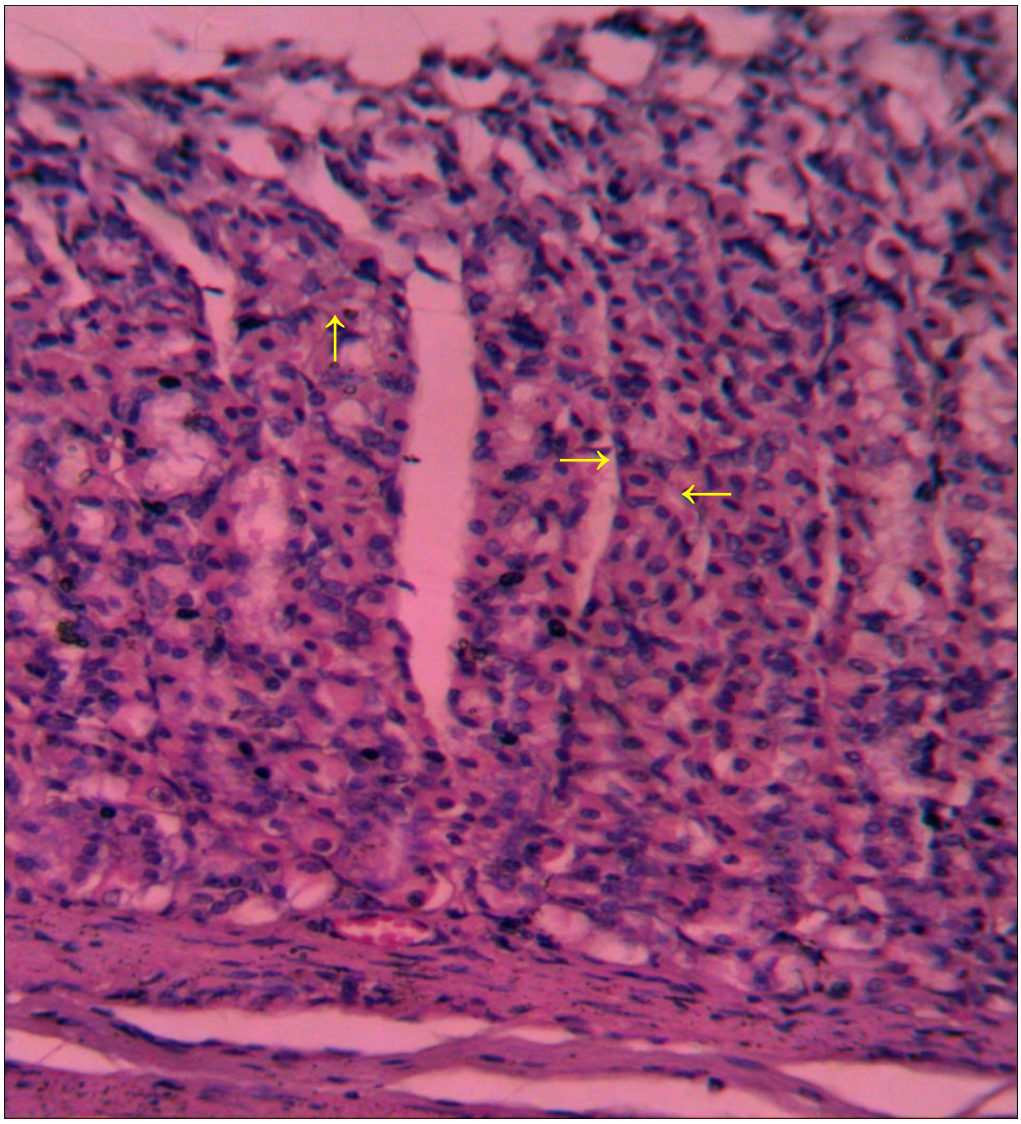

Plate 4: Photomicrograph of a section of gastric mucosa from zincgluconate treatedGroup (x250; H&E) showing a preserved general outline of the mucosa. However, some area of focal necrosis of cells were seen (yellow arrow) characterized by darkening of nuclei.

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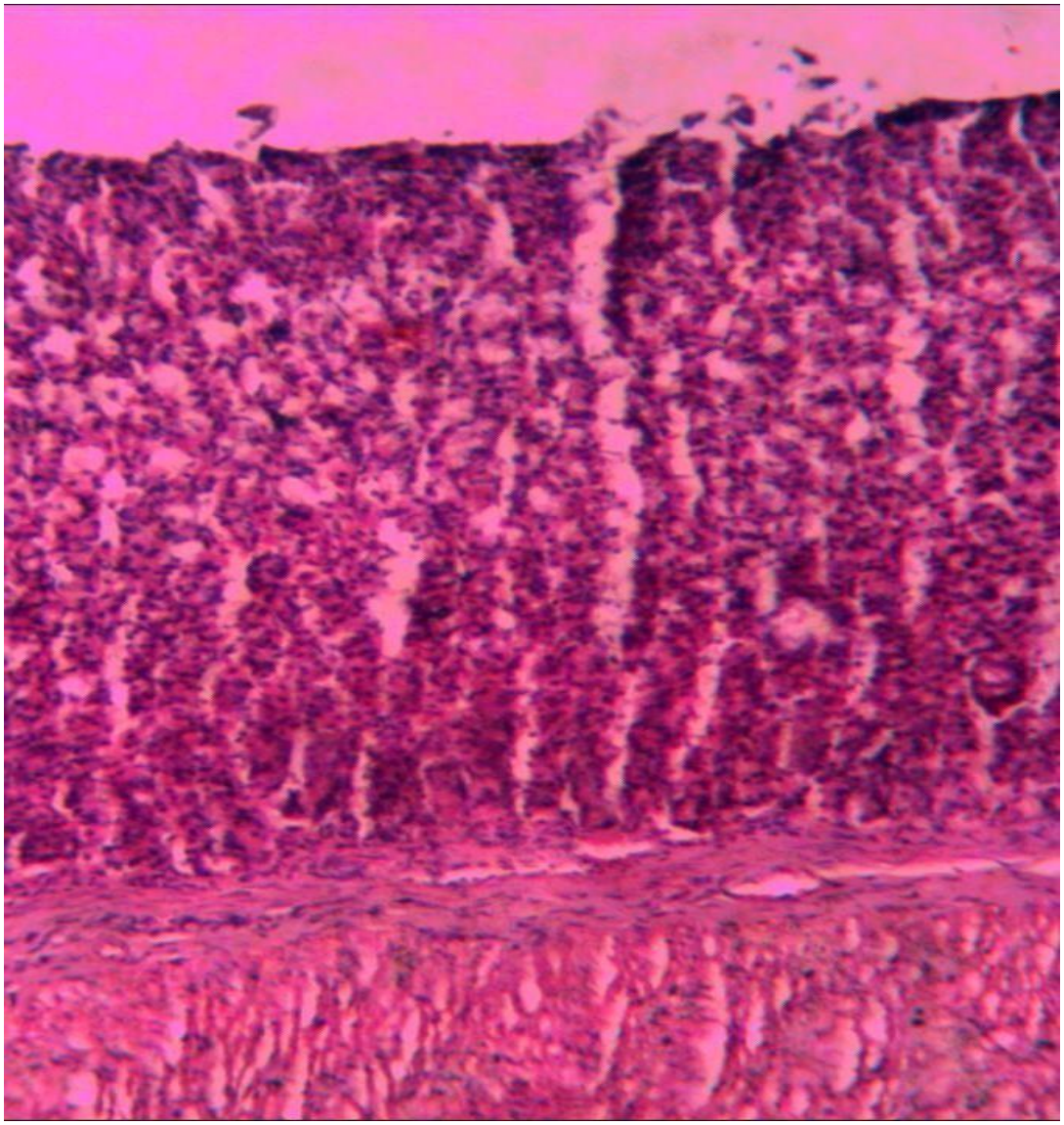

Plate 5: Photomicrograph of a section of gastric mucosa from NSO+ZINC treated group (x250; H&E) showing a general outline of the mucosa, the size of the mucosa appear preserved but not the integrity.

CHAPTER FIVE

5.0 Discussion

The preliminary Phytochemical screening of *Nigella sativa* oil revealed the presence of carbohydrates, unsaturated steroids, triterpenes, unsaturated sterols, cardiac glycoside and flavonoids. Ethanol induced gastric lesions; impair gastric defence factors such as mucus and mucosal circulation (Kushima*et al*., 2005).The administration of absolute ethanol produced 100% gastric mucosal damage leading to increased neutrophils infiltration into the gastric mucosa. Activation and infiltration of neutrophils appeared to be involved in the initial process of formation of the lesion (Jainum*et al*., 2006). It causes severe oxidative stress in gastric tissue manifested as peroxidation, increased malondialdehyde and decreased gastric gluthathione (Rao *et al.*, 2008).

In the present study, group treated with *Nigella sativa* oil, showed a reduction in the number of lesions with a preventive index of 48.8%, compared to the control group. The protective effect of the *N. sativa* oil observed in this study may be attributed to the presence of thymoquinone in the oil which is believed to improve the antioxidant status due to an increase in mucin contents of gastric mucosa (Saleh *et al*., 2000*).*This finding of the present study agreed with the result obtained by Kanter*et al*., (2005) who showed that *N. sativa* oil inhibits free radical generation and increases serum level of antioxidants. It was also reported that the *Nigella sativa* oil is involved in the endogenous generation of prostaglandins, responsible for maintaining the cellular integrity of gastric epithelium, thus playing a physiological role in protecting gastric mucosa (Robert *et al*., 1983). This protective effect of *N. sativa* demonstrated in this study is also in agreement with the studies of El-Dakhakhay*et al*., (2000) showed that NSO has a protective action against ethanol-induced ulcer in rats. Studies have shown that the protective effect of NSO against ethanol-induced ulcer may be explained by different mechanisms. First of all, the increased glutathione

level in gastric mucosa by two different ways. Firstly, through a glutathione depletion, gastric damage is caused (Jimmy *et al.,* 1997) and the increase in glutathione level caused a decrease in the ethanol induced gastric damage as reported by (Mutoh *et al.,* 1990). Secondly, glutathione being a cofactor in some steps of PGs synthesis, so it will help the conversion of PGG2 to PGH2 and the subsequent conversion to PGE2 (Chan *et al.,* 1975; Shen, 1979). This will lead to formation of different protective prostaglandins. Prostaglandin synthase is being nearly incapable of synthesizing PGE2 after depletion of glutathione from the medium (Wallach and Daniele, 1971). This postulation of increased PGE2 by NSO is in agreement with the study of (ElSayed, 1998) who found the treatment of normal and sensitized animals with NSO provoked a marked increase in PGE2 in the perfused guinea pig lung. Prostaglandins have an established role in the protection of gastric mucosa against different types of gastric lesions.

Results obtained for the volume of gastric juice secretion, showed that *N. sativa* oil treated group significantly reduced the volume of gastric juice, titratable acidity and acid output. The possible explanation for this result may be that the oil neutralized the hydrochloric acid, excessively secreted into the stomach; the neutralization of acid secretion in the stomach has already been reported to accelerate healing (Moneys *et al*., 1986). Increased production of hydrochloric acid in the ulcerated condition might be a consequence of increased permeability of the mucosa, which is an important process in the development of ulcer (Moneys *et al*., 1986). Zinc is an important element for metabolism, growth and tissue healing (Kadakis*et al*., 1992). Some clinical and laboratory studies have shown the effect of zinc component in the treatment and prevention of gastric ulcer (Santos *et al.*, 2004).The group treated with zinc gluconate demonstrated a significant reduction in the number of lesions with a preventive index of 53.6%, compared to the control group. As regards gastric secretion, the zinc gluconate treated group showed a slight

increase in volume of gastric juice, titratable acidity and acid output compared to the control group. This result is in agreement with previous work, reported by Bandyopadhay, S.K. (1997) that pre-treatment with zinc gluconate10 mg/kg body weight orally for three consecutive days, protected against alcohol induced gastric epithelial damage and in contrast with decreased acid output in rats.

The possible reason for that was zinc has been shown to inhibit free necrotic factor translocation to the nucleus, thus preventing pro-inflammatory cytokines gene expression (Aggarwal, 2002). Although zinc may not directly interact with oxidant species, it exerts antioxidant properties indirectly by stabilizing otherwise vulnerable cell membrane and is a co-factor of antioxidant enzymes, especially superoxidase dismutase and metalloproteinase, which scavenges the free radicals (Krazel, 2007). Several mechanisms have been proposed to explain the potential antioxidant effects of zinc. These includes its structural role as co-factor of SOD1 and SOD3, zinc protects sulfhydryl groups from oxidative damage, binding directly to them and thus, reducing their reactivity and preventing intra-molecular disulphide formation. Zinc contributes to the inhibition of ROS production, competing with transition metals, especially with Fe, for chelation by the organic ligand cysteine, preventing cysteine-bound Fe from transferring electrons to oxygen and producing OH (Bray and Bettger, 1990). Other antioxidant actions of Zinc have been suggested. For example, protection against vitamin E depletion, since zinc adequate diet is important for good intestinal absorption of vitamin E in rats (Kim *et al.*, 1998). Finally, zinc may be implicated in the maintenance of tissue concentration of metallothioneins, scavengers of free radicals, since they are inducible by zinc administration (Cousins, 1985).

The group treated with absolute ethanol plus *Nigella sativa* oil and zinc gluconate conferred a more significant reduction in the number of gastric lesions with a preventive index of 63.4%..The significant increase in the protective effects could be attributed to the combined antioxidant properties of thymoquinone in *N. sativa* oil and zinc gluconate as free radical scavengers in ethanol-induced gastric ulcer in rats. The *N. sativa* oil and zinc gluconate treated group have shown slight decrease in the volume of gastric secretion, titratable acidity and acid output when compared to the control group, but combined treatment offered better mucosal protection and decreased gastric secretions. The histopathological study showed that the control group, treated with distilled water revealed a normal architecture of the gastric mucosa. The ethanol-treated group revealed disruption in the gastric mucosa and enlargement of gastric pits and infiltration by inflammatory cells. While *N. sativa oil* and zinc-treated groups, showed general outline of gastric mucosa with the size of the mucosa appeared preserved. This histopathological result further confirmed the cytoprotective ability of *Nigella sativa* oil and zinc gluconate on gastric mucosal damage induced by ethanol.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendation

6.1 Summary and conclusion

The result obtained from this study showed the gastro-protective effects of *N. sativa* oil and zinc gluconate on ethanol-induced gastric mucosal damage in Wistar rats. It was revealed from the study that zinc gluconate conferred more protection to gastric mucosa than *N. sativa oil* treated group when administered separately. Nevertheless, combination of both zinc gluconate and *N,*

sativa oil provided a better gastro-protective effect than each of them administered singly. With regard to gastric secretion, the study revealed a significant decrease in the volume of gastric secretion;titratable acidity and total mean acid output in the group treated with zinc gluconate plus *N. sativa* oil, *N. sativa* oil group and zinc gluconate respectively. The preliminary phytochemical screening of *N. sativa oil* revealed the presence of carbohydrates, unsaturated steroids, triterpenes, unsaturated sterols, cardiac glycoside and flavonoids. In conclusion, both *Nigella sativa* oil and zinc gluconate administration provided gastric mucosal protection and could be used to manage the gastric ulcer disease.

6.2 Recommendations

Based on the finding of the study, the following may be recommended

1. Further experiment with different doses of *Nigella sativa oil* for similar study to assess its long-term effect on gastric ulcer.
2. Consumption of food rich in zinc locally should be encouraged to help improve the management of gastric ulcer.

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