MORPHOLOGIC AND MORPHOMETRIC STUDY ON ANTI-ULCEROGENIC EFFECTS OF SELECTED AFRICAN NIGHTSHADES (*Solanum nigrum* L.) GENOTYPES IN THE RAT

By:

Dr. Pauline Mbeere Mureithi, (BVM)

Department of Veterinary Anatomy and Physiology

Faculty of Veterinary Medicine

University of Nairobi

A thesis submitted in partial fulfillment of the Requirements for the Award of a Master of Science Degree in Veterinary Anatomy and Cell Biology of The University of Nairobi

2020

DECLARATION

This thesis is my original work and has not been submitted for a degree in any other university.

Signature……..Date……..……

This thesis has been submitted with our approval as university supervisors:

1. Dr. Boniface M. Kavoi (BVM, PGDE, MSc, PhD)

Department of Veterinary Anatomy and Physiology

Faculty of Veterinary Medicine, University of Nairobi

Signature……Date……

2. Prof. Mbaabu Mathiu (BVM, MSc., PhD)

Department of Veterinary Anatomy and Physiology

Faculty of Veterinary Medicine, University of Nairobi

Signature  Date……16th November 2020..

3. Prof. Cecilia M. Onyango (BSc, MSc, PhD)

Department of Plant Science and Crop Protection

Faculty of Agriculture, University of Nairobi

Signature  Date……16th November 2020..

DEDICATION

I dedicate this work to:

- My mother Grace Wangare and late dad Francis Mureithi, who ignited in me a thirst for education and supported me throughout the course of my studies.
- My husband Lewis Ndegwa and children Chelsea and Nathaniel who stood by me and encouraged me every step of the way.
- My parents-in-law Alice Wangare and David Macharia for their immense support and encouragement while carrying out this project.
- All my brothers and sisters for being ever so loving and supportive.

ACKNOWLEDGEMENT

I express my heartfelt gratitude to my supervisors: Dr. B. M. Kavoi, Prof. P. M. Mathiu and Prof. C. M. Onyango for their guidance and immense support during the course of my study. Their wisdom and technical knowhow contributed greatly to this work and their encouraging words pushed me to keep going.

I appreciate the following members of staff in the Department of Veterinary Anatomy and Physiology: Peter Maguta, Robert Tsuma, Francis Okumu, Peter Kiguru, Samuel Kamonde, Amos Tangai and Gabriel Keya, as well as Madam Sarah Mungania and Mr. Martin Inyimili in the Department of Human Anatomy, for their technical support during my laboratory work. I appreciate my fellow graduate students; Drs Loice Mureithi, Philip Onyono and Samuel Nderitu for their invaluable moral support and for making the journey interesting.

I'm eternally grateful to my mentor Dr. D. K. Mwangi and to Prof. S. G. Kiama for helping me secure a scholarship. My appreciation also goes to RUFORUM for additional funding.

Finally I thank God for His care and protection this far and for enabling me to reach this milestone.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

GP	Glandular portion
GU	Gastric ulcer
PAS	Periodic acid Schiff
NSAIDs	Non-steroidal anti-inflammatory drugs
GI	Gastrointestinal
CT	Connective tissue
MN	Mucous neck
Hcl	Hydrochloric acid
Bwt	Body weight
C.I	Confidence interval
MeOH	Methanol
DCM	Dichloromethane
MT	Masson's trichrome
H & E	Hematoxylin and eosin
I.p	Intraperitoneal
MaUI	Macroscopic ulcer index
MiUI	Microscopic ulcer index

ROS	Reactive oxygen species
COX	Cyclooxygenase

LIST OF APPENDICES

PUBLICATION

Pauline M. Mureithi, Boniface M. Kavoi, Cecilia M. Onyango, Mbaabu P. Mathiu (2020). Antiulcerogenic Effects of Selected African Nightshades (Solanum nigrum Linn.) Genotypes on the Rat Stomach: A Morphologic and Morphometric Study. **International Journal of Morphology 38(3):**940 - 943

ABSTRACT

Gastric ulcers are a common cause of morbidity and mortality. A gastric ulcer is defined as a peptic ulcer restricted to the gastric mucosa, induced by exposure to excessive hydrochloric acid as well as excessive pepsin activity. It leads to impaired gastric function and pain. Conventional drugs used to manage this condition have toxicity as a common side effect leading to various undesirable effects, which appear to limit the use of these drugs thereby activating research for alternative drugs. African nightshades (*Solanum nigrum* L.) has been used traditionally as a herbal cure for gastric ulcers in different parts of the world. The objective of this study was to analyze, through morphologic and morphometric means, the anti-ulcerogenic activity of three *S. nigrum* genotypes namely, *S. scabrum, S. sarrachoides* and *S. villosum* genotypes found in Kenya on the rat stomach.

Aqueous extracts of the three *S. nigrum* genotypes were obtained at the vegetative stage and subjected to phytochemical screening. The crude extracts were then administered to three groups of Wistar rats 30 minutes before administration of 1ml ethanol to induce ulceration. A negative control group was given distilled water orally, while a positive control group was given 1ml ethanol orally. Tissues were harvested from the stomach antrum and examined grossly, then processed for examination under light microscopy using Hematoxylin and Eosin, Periodic Acid Schiff and Masson's Trichrome staining methods.

The phytochemical testing revealed the presence of: terpenoids, tannins, saponins, flavonoids as well as glycosides. The three *S. nigrum* genotypes exhibited antiulcerogenic effects. *S. scabrum* showed the highest ulcer inhibition score of 76.4%, followed by *S. sarrachoides* with 72.5% and

S. villosum with 63.3%. *S. nigrum* pretreated rats showed less gastric mucosal surface erosion, congestion, edema and hemorrhage.

Penetrating ulcers in *S. nigrum* pretreated rats affected only the gastric pit region of the stomach mucosa, except for those of the *S. villosum* pretreated rats, which penetrated to affect the gastric glands. *S. nigrum* pretreatment resulted in more intense staining, (compared with rats that were not treated before ulcer induction) of the mucus regions of gastric glands with PAS and of inter-glandular connective tissue with Masson's trichrome denoting less gastric damage in these animals. Microscopic ulcer index scores decreased 5.1, 3.6 and 2.4- fold in *S. scabrum, S. sacharroides* and *S. villosum* pretreated rats respectively.

Results of this work show that extracts of the three *S. nigrum* genotypes are antiulcerogenic in varying degrees with *S. scabrum* being the most effective. The observed differences in ulcer inhibition capacities of the three *S. nigrum* genotypes may be attributed to genetic factors, which reportedly influence the nature and composition of bioactive ingredients synthesized in medicinal plants. Further studies to isolate and quantify phytochemicals responsible for this activity at different stages of plant maturity are recommended.

CHAPTER ONE

1 INTRODUCTION

Gastric ulceration refers to lesions on the glandular part of the stomach mucosa induced by exposure to excessive hydrochloric acid and excessive pepsinogen activity (Sabiu *et al*., 2015). The stomach is a component of the digestive tube specialized in mechanical and chemical breakdown of food (Colville & Bassert, 2016). Like in other parts of the GIT, the wall of the stomach in mammals consists of four layers named from the inside outwards as: i) tunica mucosa comprising an epithelium, a lamina propria of loose connective tissue and a muscularis mucosae ii) tunica submucosa consisting of loose areolar connective tissue with plenty of blood vessels iii) tunica muscularis constituted by thick layers of visceral muscle fibers and iv) tunica serosa (Burkitt *et al*., 1993). There are four main types of epithelial cells that cover the stomach mucosal surface and extend down into the gastric pits and gastric glands. These include mucus-secreting cells, parietal/oxyntic cells which produce Hcl, peptic/chief cells which produce proteolytic enzyme pepsin and G (gastrin) cells that synthesize peptide hormone gastrin. Ulceration studies show the rat stomach to be uniquely susceptible to gastric lesions (Greaves, 2012), which in the current study were analyzed in the glandular region (part that is in constant contact with the highly corrosive gastric juice).

Gastric ulceration (GU) leads to impaired gastric function and pain and is a common cause of morbidity and mortality, with close to 53 million people developing gastric ulcers in the world each year (Vos *et al*., 2015). Lifestyle changes including smoking, drinking, stress and the consumption of NSADs in chronic illnesses such as arthritis are some of its predisposing factors (Moore *et al*., 2014; Kołodziejska & Kołodziejczyk, 2018). It is often associated with complications such as perforation, bleeding, blockage and if long standing, GU precipitates to

gastric cancer (Pilotto *et al*., 2003, Milosavljevic *et al*., 2011; Bhattacharyya *et al.,* 2014). Conventional drugs used to manage this condition include prostaglandin analogues, proton pump inhibitors, antacids and antibiotics in case of *Helicobacter pylori* infections. These however have toxicity as a common draw back leading to various side effects including hypersensitivity, hepatitis, hematopoietic changes and arrhythmias (Odou *et al*., 1999; Marcus *et al*., 2010). This therefore necessitates a search for anti-ulcerogenic medicines that are less toxic. Indeed, medicinal plants are highly preferred alternatives due to their easy accessibility and the fact that they are perceived to be less toxic than conventional drugs (Ernst & Hung, 2011). The use of medicinal plants to manage disease conditions dates back to human civilization (Mosihuzzaman, 2012). In developing countries, approximately four billion people (about 60% of the worldwide human population) rely on herbal products for their healthcare needs (Bodeker *et al*., 2005). The knowledge on indigenous plants and their medicinal uses has traditionally been passed down from one generation to the next (Miaron *et al*., 2004). Recently a lot of research has been carried out to validate commonly used folk medicines (Lahlou, 2013).

Solanum nigrum, commonly known as African night shade, is used as a vegetable and also as treatment for various ailments including gastric ulcers (Edmonds and Chweya, 1997: Ontita *et al*., 2016). However, scientific evidence for the use of the genotypes grown and utilized in Kenya in ulcer management is lacking. This study therefore aims at analyzing the efficacy of three *S. nigrum* genotypes namely: *S. scabrum, S. sarrachoides and S. villosum* grown in Kenya in preventing gastric ulcers.

1.1 Objectives

1.1.1 Broad objective

The overall objective of this study was to determine the anti-ulcerogenic activity of three *S. nigrum* genotypes (*S. scabrum, S. sarrachoides and S. villosum*) found in Kenya, in a rat model.

1.1.2 Specific objectives

1. To determine the phytochemical composition of the three *S. nigrum* genotypes grown and consumed in Kenya.
2. To describe the morphological differences in anti-ulcerogenic activity of the three *S. nigrum* genotypes on the rat stomach.
3. To determine the morphometric differences in anti-ulcerogenic activity of the three *S. nigrum* genotypes on the rat stomach

1.2 Null hypothesis

Solanum nigrum extracts are not anti-ulcerogenic and there are no morphological or morphometric differences in efficacy of ulcer prevention of the three *Solanum nigrum* genotypes.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Gastric ulcers

A gastric ulcer is a form of peptic ulcer that occurs on the lining of the stomach. It presents as mucosal damage, following pepsin and gastric acid secretion (Ranakrishnan and Salina, 2007; Sung *et al*., 2009). Gastric ulcer lesions are induced by an imbalance between aggressive factors, such as excessive hydrochloric acid, pepsin, bile and reactive oxygen species (ROS), and cytoprotective factors such as the mucus barrier, rapid renewal of gastric cells, enzymatic and non-enzymatic antioxidants, as well as growth factors (Bhattacharjee *et al.*, 2002; Sabiu *et al*., 2015). Common causes of this condition include: Helicobacter pylori infection, chemical injury to gastric mucosa associated with the use of acetylsalicylic acid, alcohol and non-steroidal anti-inflammatory drugs (NSAIDs), stress as it occurs during chronic illness, nutritional deficiencies, some viral infections among others (de Souza *et al*., 2011; Moore *et al*., 2014; Kadhin *et al*., 2015). NSAIDs in particular are often used in the management of musculoskeletal diseases such as arthritis, with their use being associated with gastrointestinal (GI) injury (Moore *et al.*, 2014: Velasco-Zamora *et al*., 2016).

Gastric ulcers commonly cause morbidity and mortality, with an estimated 53 million people developing peptic ulcers globally in 2013 alone (Vos *et al*., 2015). Gastric ulcer complications include bleeding, perforations, penetration and obstruction (Milosavljevic *et al*.,2011). Gastric ulcers are also precursors to gastric cancer, with various studies elucidating the mechanism by which this occurs (Correa and Houghton, 2007: Ding *et al*., 2010). These

complications lead to a lowered quality of life as well as economic loss due to the cost of treatment and time spent away from work (Knill-Jones *et al.*, 1990).

The pathogenesis of GU is multifactorial. Oxidative stress as occurs in ethanol induced GU is a major factor in the pathogenesis of gastric ulcers (Bhattacharyya *et al*., 2014). Other factors include the macrophage migration inhibition, blockade of cyclooxygenase (COX) activity, decreased synthesis of prostaglandins and high pro-inflammatory release of cytokines (Huang *et al*., 2001; Atherton., 2006; Jainu and Devi, 2006). Gastric ulceration in humans is diagnosed through a blood test for antibodies, stool test for the bacteria or for h. pylori antigens, a breath test for urea or a stomach biopsy. Confirmation is usually done by barium swallow or endoscopy (Pilotto *et al*., 2003: Najm, 2011).

2.2 Treatment of gastric ulcers

Gastric ulcers are commonly treated using a combination of drugs. Proton pump inhibitors for example ranitidine and omeprazole which reduce gastric acid secretion are often combined with antibiotics especially those known to have activity against H. pylori. Antibiotic resistance is on the rise hence this treatment method may be challenged (Wang and Peura, 2011). Other drugs used in the management of gastric ulcers include prostaglandin analogues, histamine H2-antagonists, anti-muscarinics and antacids (Loludice *et al*., 1981: Bighetti *et al*., 2005).

These drugs have associated side effects ranging from diarrhea, dizziness, hypersensitivity, hepatitis, arrhythmia, hematopoietic changes and impotence (Romero-Gomez *et al*., 1999, Odou *et al*., 1999). These adverse side effects accompanied by a high rate of GU recurrence are a common limiting factor in the use of these drugs hence the need for cheaper, more readily available drugs with little or no side effects.

2.3 Use of herbaceous plants for treatment of ailments

Herbaceous plants are commonly used all over the world for treatment of various ailments. This is because they are perceived to be less toxic, more affordable and highly accessible (Nanyingi *et al*., 2008). In developing countries, most of the population depends on herbaceous plants with an estimated 80% of the population in the Southern hemisphere relying on herbal medicine (WHO, 2003). Herbaceous plants have therefore recently received a lot of attention as possible sources of alternative drugs for various ailments with their gastroprotective activity being attributed to their ability to increase mucosal defensive factors (Awaad *et al*., 2013). Phytochemical screening has led to isolation of certain phytoconstituents of these plants, responsible for their prophylactic and curative properties, while acute toxicity studies have been used to rule out some toxic plants used in herbal medicine (Pour *et al*., 2011; Majeed *et al*., 2015).

African Nightshades (*Solanum nigrum* spp.), also commonly referred to as Black, Common or Garden Nightshades, are a group of plants in the family Solanaceae. African Nightshades are annual herbs that are either creeping/ prostate, semi erect or erect. Their leaves are ovate or lanceolate with serrated or entire margins and about 8- 15cm long. Flowers may be white or

purple. Their berries are either black, purple, orange or red in colour. (Figure 2.1). This family contains both medicinal as well as poisonous plants, and is found growing in tropical and temperate areas (Onyango, 2010; Wesonga, 2017).

Figure 2.1: A photograph of the African Nightshade (*Solanum nigrum)* plant.

Solanum is one of the largest as well as most complex genus in this family comprising of many different species. Species within this genus are of high economic value and include food plants like the potato (*S. tuberosum* L.) as well as plants cultivated for their medicinal value like bittersweet (*S. dulcamara* L.) as well as soda apple (*S. viarum* Dun.), which both produce corticosteroids (Edmonds and Chweya, 1997).

The *Solanum nigrum* complex is a source of fruits and vegetables in Kenya and other parts of the world (Schippers, 2000). In addition, it has been shown to have medicinal value and is one of the many medicinal plants shown to have gastroprotective potential (Potawale *et al*., 2008; Jagatheeswari *et al*., 2013; Majeed *et al,* 2015) and is used in Kenya to treat stomach aches and ulcers, to sooth the gum of teething babies and to treat tonsillitis (Edmonds and Chweya, 1997; Matasyoh and Mwaura, 2014; Ontita *et al*., 2016). The plant was also found to inhibit growth of cervical carcinoma in experiments on mice (Jian, 2008). The plant is also used for its antimicrobial and larvicidal activity (Rawani *et al*., 2013).

Medicinal plants grown in different ecological zones have been shown to differ in phytochemical composition (Zouari *et al*., 2014; Yao and Bo, 2016). This therefore necessitates further research in to the anti-ulcerogenic properties of the different genotypes of *Solanum nigrum* grown in Kenya.

2.4 The stomach

2.4.1 General histological structure of the stomach

The stomach is the portion of the digestive system specialized for storage as well as enzymatic and hydrolytic breakdown of food (Colville and Bassert, 2015). It is lined by either a glandular or non-glandular mucosa, or both depending on species. The wall of the stomach is made up of four layers consistent with those of the rest of the digestive tube. The innermost layer is the tunica mucosa, comprising of an epithelium, a lamina propria as well as a muscularis mucosa. The next layer is the submucosa comprising of collagen

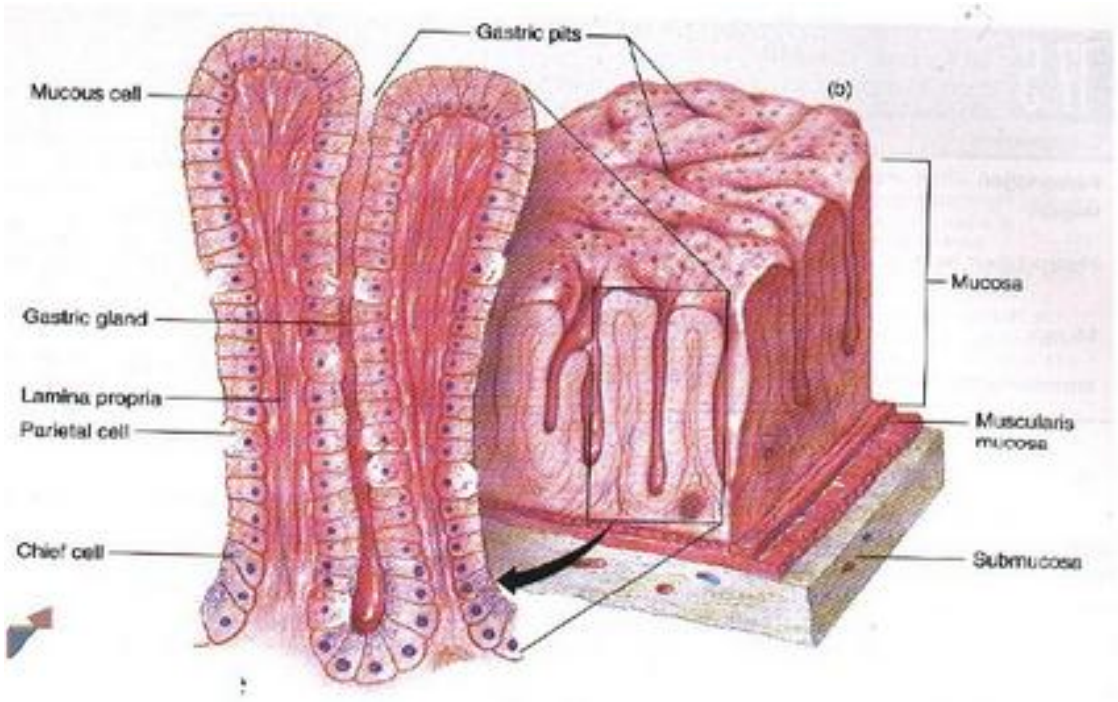
fibers, fat, blood vessels and nerve plexuses. The tunica muscularis is the third layer, which comprises the inner oblique, middle circular and an outer longitudinal layer of smooth muscles. The tunica serosa is the outermost layer and comprises mesothelium overlying a layer of loose connective tissue (CT).

2.4.2 The non-glandular region

This region has a stratified squamous epithelial lining, continuous with that of the esophagus. This epithelium is non-secretory and may be keratinized in some species depending on diet (Ghoshal and Bal., 1989). The lamina propria here is made up of elastic, reticular and collagen fibers. The junction between the glandular and non-glandular region is abrupt, with the epithelium transitioning to a tall columnar type.

2.4.3 The glandular region

The mucosa in this region is characterized by extensive folds known as rugae (Chivers and Hladik., 1980). The surface has small invaginations known as gastric pits, that are continuous with and receive the secretions of gastric glands. This mucosa is lined by columnar epithelial cells. Majority of the lamina propria is occupied by gastric glands with only a few CT fibers and cells in between. The muscularis mucosa is quite thick here and is made up of an inner and outer circular layer and a middle longitudinal layer. Tiny bundles of smooth muscle fibers extend into the mucosa, running through the CT fibers between the gastric glands. The glandular mucosa has three regions, each named according to the type of glands present. These are the cardiac gland region, the fundic gland region and the pyloric gland region.



2.2: A schematic representation of gastric glands and the associated cell types (source: http://zoologybox.blogspot.com/2015/10/define-gastric-gland.html)

2.4.4 The cardiac gland region

The cardiac gland region lies in a narrow strip at the junction between the glandular and non-glandular mucosa. Cardiac glands are tubular, branched and coiled glands that open into the gastric pits. The body of the gland is fairly short with a wider lumen than that seen in the fundic or pyloric glands. The mucous secretory epithelium is cuboidal with nuclei located at the basal part of these cells. Parietal cells may occupy the junction of the cardiac gland and fundic gland regions.

2.4.5 The fundic gland region

The fundic gland region is well developed in most animal species (Kondo., 1966). Fundic glands are tubular, branched and straight glands which extend to the muscularis mucosa. The glands have a neck portion, an elongated body, and a slightly enlarged blind end. Four functionally and structurally distinct cell types make up the secretory epithelium of fundic glands (Eurell and Frappier, 2013). These include the mucous neck (MN) cells, chief cells, parietal cells and argentaffin cells.

The MN cells are found in the neck of the gland among the parietal and chief cells (Eurell and Frappier, 2013). They are typical mucous cells with the flat nucleus located towards the cell base. They appear similar to the surface cells but their cytoplasm is more basophilic. In addition, when treated with Periodic Acid Schiff (PAS), the MN cells give a uniform intensely positive reaction, while the surface cells have PAS – positive material in the upper two thirds of the cell only (Eurell and Frappier, 2013).

The chief cells (zymogen cells) are the most numerous of the gastric gland cells (Tseng *et al*., 1987). They are cuboidal or pyramidal. Their spherical nucleus is located at the base of the cell. Spaces where zymogen granules occupy in the living state appear frothy. Their basal area has a well-developed rough endoplasmic reticulum, giving a basophilic staining reaction. Chief cells secrete pepsinogen which is transformed into pepsin by hydrochloric acid (Hcl). Parietal cells are more numerous than chief cells. They are pyramid shaped and are located peripheral to the chief cells. They have a spherical nucleus with an eosinophilic cytoplasm. They produce Hcl.

Argenataffin cells are moderately abundant in the fundic gland as single cells wedged between the basement membrane and the chief cells (Tseng *et al*., 1987). These can only be demonstrated using silver stains or in electron micrographs. They produce serotonin which stimulates vasoconstriction and smooth muscle contraction.

2.4.6 The pyloric gland region

Pyloric glands are branched, coiled and relatively short compared to other gastric glands (Kondo., 1966). The gastric pits here are relatively deeper than those in the cardiac and fundic gland regions. The cells are mucous cells and have a flat nucleus located at the base of the cell. At the pyloric -duodenal junction, the submucosal intestinal glands are found in the submucosa of pyloric region. The middle circular muscle layer also thickens, forming the pyloric sphincter.

2.5 The rat stomach

The rat stomach has both a glandular and a non-glandular region (Ghoshal and Bal, 1989). The non-glandular fore-stomach has rumen-like mucosal folds covered with keratinized stratified squamous epithelium (Uehara *et al.*, 2018). It is translucent, thin walled and serves as a storage for ingested food (Vdoviaková *et al*., 2016). The glandular portion (corpus), is characterized by simple columnar epithelium. Gastric glands comprise primarily of parietal cells and chief cells, although argentaffin-like cells have been described occasionally. The pyloric region has simple columnar epithelium beneath which the pyloric glands lie (Ghoshal and Bal, 1989: Suckow *et al*., 2005).

The glandular portion is separated from the non-glandular portion by a fold of the non-glandular mucosa known as the "limiting ridge". The ridge overlaps a deep groove known as the "gastric groove" (Luciano and Reale, 1992). Brush cells found at this groove are thought to play a role in the transfer of food from the non-glandular to the glandular portion of the stomach (Eberle *et al*., 2014). The surface of the glandular stomach has a mucous layer that is 10-20 times thicker than its surface epithelium that serves to protect it from injurious factors (Uehara *et al*., 2018). In studies on gastric ulceration (Robert *et al*., 1979; Shetty *et al*., 2000; Khazaei and Salehi, 2006) the glandular portion of the rat stomach has been used since the non-glandular portion does not appear to have any lesions on gross examination.

2.6 Physiology of gastroprotection

The gastric mucosa acts as a barrier that protects deeper gastric tissues from the injurious effects of gastric acid as well as ingested irritants. Various physiological functions protect the gastric mucosa from chemical injury. These include mucous secretion, rich mucosal blood supply, rapid renewal of mucosal cells, enzymatic and non-enzymatic antioxidants, growth factors among others (Flemström and Turnberg, 1984; Bhattacharjee *et al*., 2002). These are mostly mediated by the activity of prostaglandin cyclooxygenase (COX), nitric oxide, peptides as well as other neural mechanisms (Konturek., 1990: Peskar, 2001). Prostaglandins are responsible for increased mucosal blood flow thus increasing mucus production and bicarbonate anion production (Brzozowski *et al*., 2004). The bicarbonates in turn neutralize gastric acid.

2.7 Induction of ulcers in experimental animals

Various methods are used to induce ulceration in experimental animals. These include both physical and chemical methods. Chemical methods involve use of drugs such as aspirin, diclofenac and indomethacin, histamine, serotonin, acetic acid and ethanol (Kang *et al*., 1996: Pandian *et al*., 2002: Sannomiya *et al*., 2005). Physical methods include swimming/water immersion stress, cold restraint and surgery (Grover *et al*., 2001). For this study, 80% ethanol was used to induce ulcers in the experimental animals.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Plant material collection and extract preparation

Three genotypes of *S. nigrum* namely: *S. scabrum, S. sacharroides* and *S. villosum*, were collected from the Kenya Agricultural Research Institute (KARI), Muguga, which is located 27km North West of Nairobi, Kenya, and lies 1°13_S, 36°38_ E, and is 2096m above sea level. The rainfall in Muguga is bimodal and is between 900mm to 1000mm annually. Long rains are experienced between March to June and they total about 550mm, while short rains fall between mid-October and December and total about 400mm. The temperature ranges between 7°C to 20°C with a mean of 15ºC. The area has well drained soil which is reddish brown to dark red in colour (FAO, 2006). The plant genotypes were authenticated by a botanist at the School of Biological Sciences, University of Nairobi, Botany section, and voucher specimens archived in the herbarium.

3.2 Sample preparation

The leaves were obtained at vegetative stage from the various genotypes and were air dried at room temperature (20-25 ^{o} C) for a week and were constantly turned to prevent fungal growth (Mishra *et al*., 2012). The leaves were then ground into a powder to pass through a 2mm sieve. For each genotype, 100g of the powdered leaves was boiled in 500ml of water for 15 minutes. The aqueous extract was then sieved, freeze dried using a Virtis Freeze Dryer and stored at -20 ^{o}C for further use.

3.3 Phytochemical analysis

Phytochemical analysis was carried out as described in Maobe *et al.,(*2013) and the samples were analyzed for terpenoids, flavonoids, tannins, saponins and glycosides.

Determination of terpenoids

About 1g of plant extract was placed in a test tube and 10mls of methanol added then filtered. 2ml of chloroform was added to 5mls of the filtrate. 3ml of sulphuric acid was added and observed for colour change. Formation of a reddish brown colour indicated presence of tarpenoids in the extract

Determination of flavonoids

About 2g of the extract was placed into a test tube containing 10mls of distilled water. 5ml dilute ammonia solution was added to a portion of the aqueous filtrate. 1ml of concentrated sulphuric acid was then added and observed for colour change. Formation of a yellow colour indicated presence of flavonoids in the extract.

Determination of saponins

This was done by placing 0.5g of the plant sample in a test tube. 3 ml of distilled water was added and heated in a water bath while shaking. Frothing indicated the presence of saponins.

Determination of tannins

2.0 ml of the extract of the sample was placed in a test tube and an equal volume of water was added. 2 drops of 10% Sodium hydroxide (NaOH) was added. A green colouration indicated presence of tannins.

Determination of glycosides

About 2ml of the extract was dissolved in a mixture of 1ml glacial acetic acid and 1 drop of 5% ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A reddish brown ring at the interface indicated the presence of glycosides.

3.4 Morphological determination of differences in anti-ulcerogenic activity of the three *S.nigrum* genotypes on the rat stomach

3.4.1 Experimental animals

Adult male Wistar rats 6-8 weeks old, with a body weight (bwt) range of 220-250g, were used for this study (Koolhaas, 1999). They were kept under standard housing conditions (temp 25^{o}C, 12h day/night cycle, 60% relative humidity) with food and water provided *ad libitum*. The animals were allowed 10 - 14 days to acclimatize to the housing conditions before the experiments were carried out. All experiments were performed according to the guidelines of the Animal Use and Care Committee of the Faculty of Veterinary Medicine, University of Nairobi (Ref: FVM BAUEC/2016/120).

3.4.2 Acute toxicity studies

This was performed according to OECD guidelines (OECD 420, 2000). Five rats were given a single dose of 2000mg/kg bwt of the extract dissolved in normal saline orally. The animals were continuously observed for the first 4 hours for behavior, neurologic and autonomic profiles. They were also observed after 24 hours for any deaths and for 14 days for hair coat changes.

3.4.3 Drug administration

The doses used were based on the acute toxicity studies. A total of 36 rats were used for this study. This sample size was determined using the formula described by Martin *et al*., (1987):

$n = (z/p)2\pi(1-\pi)$ where:

n = the required sample size

p = the desired maximum discrepancy (i.e. ±5%)

π = the population proportion

z = the appropriate z value from the normal distribution for the desired confidence interval (C.I). Therefore, for a 50% population proportion at 95% C.I,

$n = (1.96/0.05)^2 0.5(1-0.5) = 384$

However for ethical considerations and as per earlier studies (Shetty *et al.*, 2000: Jainu and Devi, 2004), this sample size was reduced to 36 rats which were proportionately assigned to six groups of n = 6. The rats were starved for 24 hours, but water was provided *ad libitum*. Group 1 was the negative control and received normal saline (5ml/kg) while group 2 was the positive control and received 1ml of 80% ethanol. Groups 3 – 6 were the test groups and received 500mg/kg of the extract from the three different *solanum nigrum* genotypes followed in 30 minutes by 1.0 ml of 80% ethanol orally (Bighetti *et al*.,2005). The rats were then sacrificed 1 hour following ethanol administration using lethal intraperitoneal doses of pentobarbital sodium (140mg/kg bwt). The stomachs were then removed from the abdominal cavity, opened along the greater curvature and gently rinsed in physiological saline.

3.5 Morphometric determination of differences in anti-ulcerogenic activity of the three *S. nigrum* genotypes on the rat stomach

3.5.1 Macroscopic morphometry

The stomachs of all the groups were examined by three pathologists independently for the presence of the following lesions: hyperemia, edema, hemorrhages, erosions and ulcers. The summative length (mm) of the lesions was measured under a stereo microscope. Five petechial lesions were considered as 1mm of ulcer (Alkofahi and Atta, 1999).

The number of ulcers as well as their severity were recorded on a scale of 0 – 5, a modification of the method described by Shetty *et al*, (2000) where:

0 = No ulcer

1= less than 5 slight lesions

2= more than 5 slight lesions

3= 1-3 hemorrhagic bands, <5 mm in length

4= 1-3 hemorrhagic bands >5 mm in length

5= diffuse hemorrhagic lesions

The Macroscopic Ulcer Index (MaUI) was then worked out following Vogel and Vogel, (1997) formulae

$$\mathbf{MaUI = UN + US + UP \times 10^{-1}}$$

Where,

UI = Ulcer index

UN = Average number of ulcers per animal

US = Average number of severity score

UP = Percentage of animals with ulcers

The percentage inhibition of ulceration was calculated as:

$$\textbf{\% Inhibition of ulceration} = \frac{\text{(Ulcer index positive control – Ulcer index Test)}}{\text{Ulcer index of negative control}} \times 100$$

3.5.2 Microscopic morphometry

Histomorphometric evaluation was carried out following standard methods (Carleton, 1980; Kavoi et al., 2010). The stomachs, after being analyzed for gross anatomical changes, were fixed in 10% formaldehyde. The glandular portion was cut into strips containing all the layers of the stomach then processed for paraffin wax sections by washing them overnight in running tap water followed by, dehydration in an ascending concentrations of ethanol (50%, 70%, 80%, 90%, 95% and absolute), for 2 hours in each solution with two changes at the level of the absolute alcohol. The samples were cleared in 2 changes of methyl benzoate, for 2 hours and infiltrated in a paraffin wax oven maintained at 58-59^{0}C, overnight. They were then embedded in paraffin wax. The wax blocks were attached to labeled wooden blocks. Tissues cut using a rotary microtome at 6µm were stained by H&E method (Carleton, 1980), Masson's trichrome staining method as well as PAS staining method. The histology images were sampled on a Leica DRM light microscope and transferred to a monitor where an image analyzing software was used to measure the depth of the ulcers. The images that were analyzed were selected in a random systematic sampling manner and a minimum of seven fields analyzed on each slide.

Microscopic Ulcer Index (MiUI) was obtained on a scale of 0 - 3 as described by Khazaei and Salehi (2006). Where:

0= Normal tissue

1= Local damage to gastric pit cells

2= Local damage to gastric glands

3= Deep damage to gastric glands

A mean index was calculated as follows:

MiUI = (number of lesions 1) + (number of lesions 2) × 2 + (number of lesions 3) × 3

3.6 Occupational health

During the study proper biological and chemical safety protocols were observed by the animal handlers and laboratory workers. Personnel wore personal protective equipment such as gloves, dust masks, lab coats and closed shoes. Proper use of laboratory equipment was ensured at all times and food and drinks were not allowed into the lab. A fume hood was used when working with poisonous chemicals and all chemicals were stored in locked safety cabinets, in clearly labeled containers.

3.7 Safe disposal of carcasses, sharps and chemicals

Animal carcasses were placed in plastic bags which were then incinerated. Sharps (surgical blades, needles and broken glass), and chemical waste were disposed by placing in appropriate labeled containers and handed to the university disposal authority .

3.8 Data analysis

Quantitative data was subjected to one way analysis of variance (ANOVA) and Dunnett's pair wise test was used to compare differences between treatments. Results were considered significant at 95% confidence interval and expressed as mean ± standard deviation (SD). Qualitative data were analyzed using Graphpad Prism 5.

CHAPTER FOUR

4 RESULTS

4.1 Phytochemical composition of plant extracts

The aqueous extracts of the three *S. nigrum* genotypes were found to contain saponins, tannins, flavonoids and terpenoids

Results obtained from phytochemicals screening of the aqueous extracts of the three *S. nigrum* genotypes are presented in Table 4.1.

Table 4.1: Phytochemical composition of aqueous extracts of the three *S. nigrum* genotypes.

	S. villosum	***S. sarrachoides***	***S. villosum***
Glycosides	-	-	-
Saponins	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+

Key: + present

- Absent

4.2 Acute toxicity findings

Oral administration of a single dose of 2000mg/kg bwt of the aqueous extract was found to have no adverse effect on the animals. No behavior or hair coat changes were observed, and no deaths were recorded. These extracts were therefore considered safe.

4.3 Morphologic findings

4.3.1 Macroscopic findings

Lesions within the stomach were restricted to the glandular portion (GP) of the stomach. They included edema, hyperemia, hemorrhage as well as linear necrotic lesions. The linear necrotic lesions ran parallel to the long axis of the stomach (Fig. 4.1).

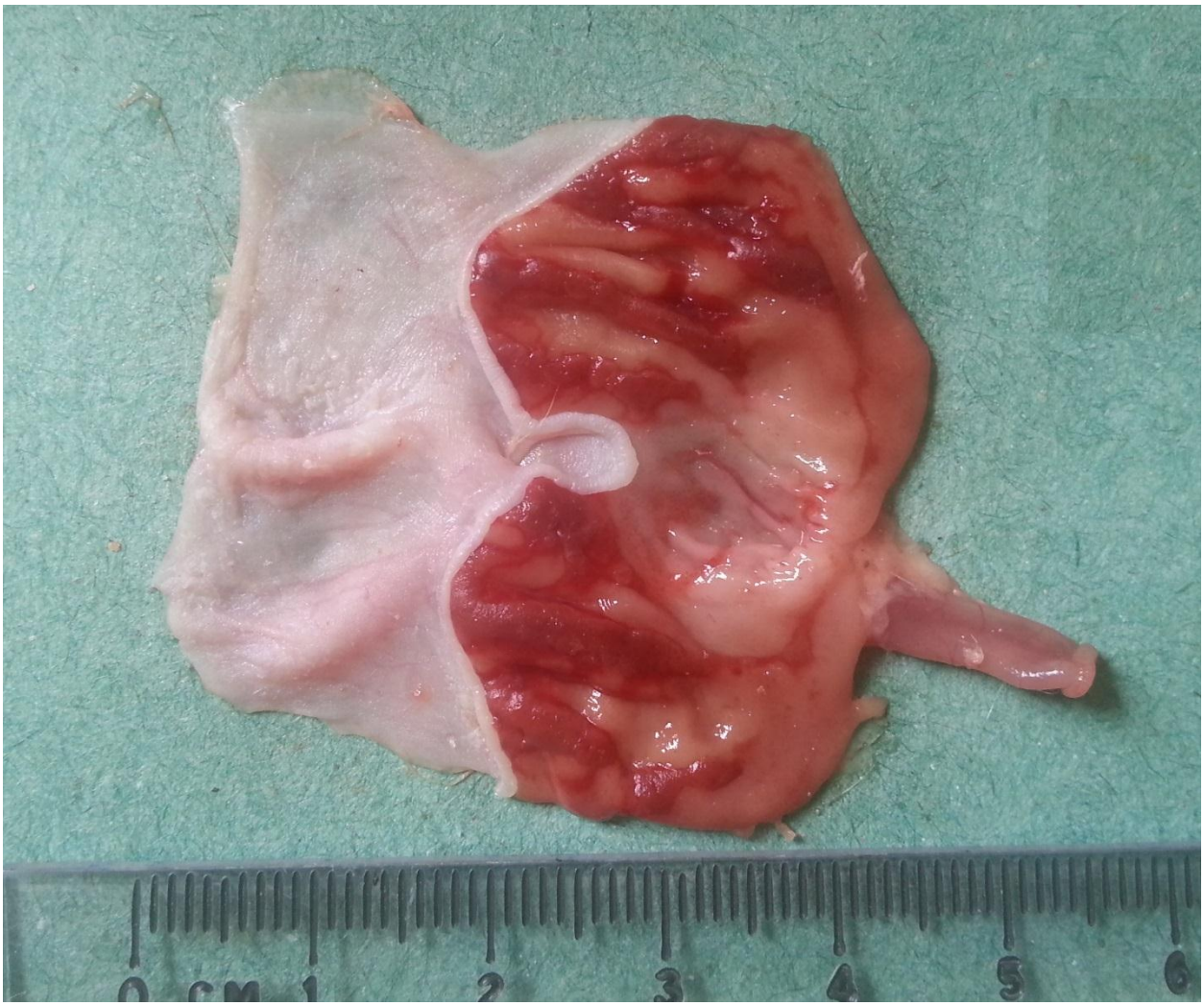


Figure 4.1: Linear necrotic lesions within the glandular portion of a rat stomach following oral administration of absolute alcohol.

A comparison between a dose of 300mg/kg and 500mg/kg bwt revealed less ulceration at the later dose, hence this was the preferred dose.

Positive control rats had the most extensive lesions. Positive control rats exhibited closely spaced ulcerative bands on the mucosal surface (fig. 4.2). The three *Solanum nigrum* genotypes differed in their efficacy of gastroprotection, with the *S. Scabrum* having the highest efficacy, followed by the *S. sarrachoides* and *S. villosum* genotypes respectively.

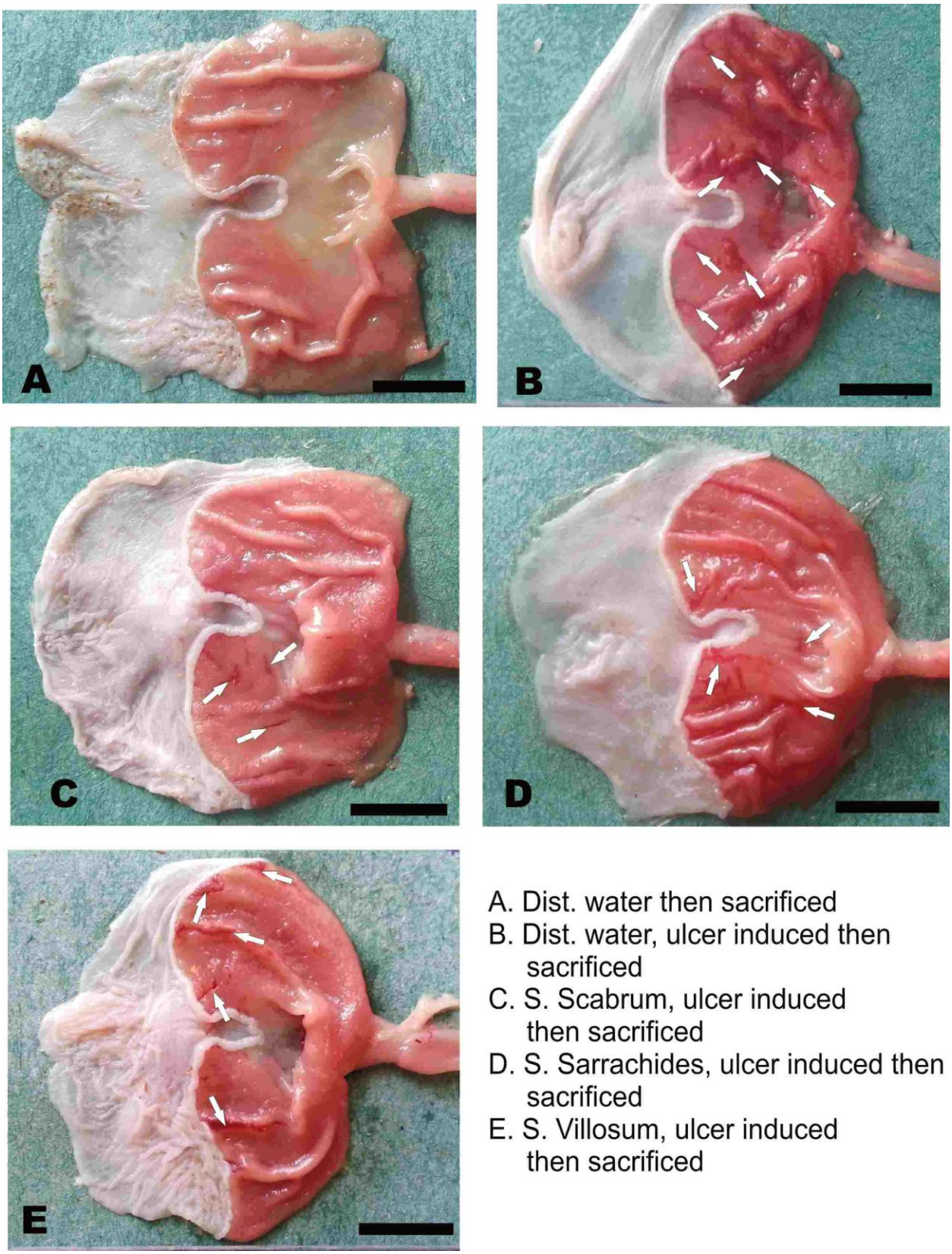


Figure 4-2 Macrographs of stomachs of rats from the five different treatment groups.

Notice the absence of lesions in A and the massive gastric ulcerations (arrows) in B. There is evidence of ulcer protection by all *S. nigrum* genotypes as revealed by the marked reduction in number of ulcer areas (arrows), with the most effective extract being *S. scabrum* (C) followed by *S. sarrachoides* (D) and then *S. villosum* (E). Bar= 1cm.

4.3.2 Light Microscopic findings

The GP of the rat stomach had the typical layers including the tunica mucosa, consisting of epithelial lining, lamina propria and muscularis mucosa, the submucosa, the tunica muscularis consisting of the inner oblique, middle circular and outer longitudinal smooth muscles and the serosa. The mucosa had distinct glands, showing pits, neck and body regions, with the muscularis mucosa extending in between the gastric glands (figure 4.3).

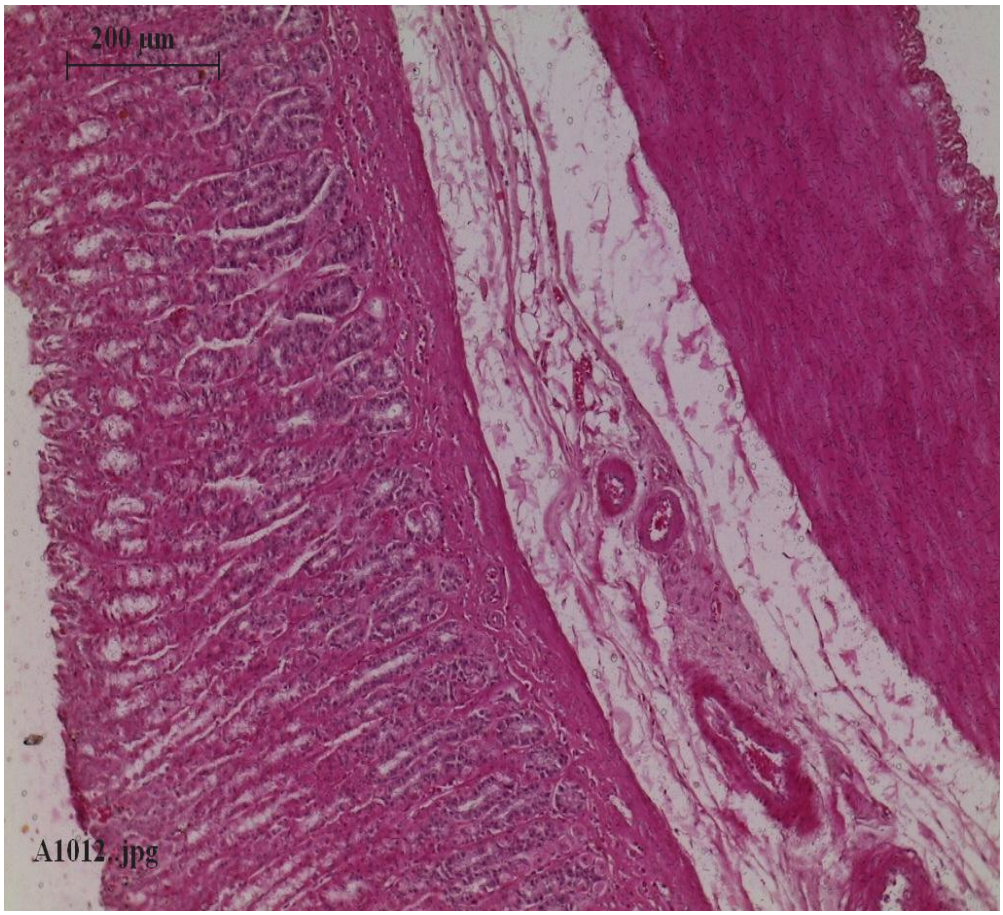


Figure 4-3: Microscopic view of the normal rat stomach wall as appeared in the negative control animals.

Microscopic lesions included vasocongestion, loss of surface epithelium, dilated gastric glands and extravasation. The lesions were seen to penetrate through the gastric pits and glands, terminating at the tunica submucosa. Lesions were notably fewer in rats pretreated with the three *S. nigrum* extracts, affecting only the gastric pits, except in rats pretreated with the *S. villosum* extract where the lesions extend into the gastric gland region.

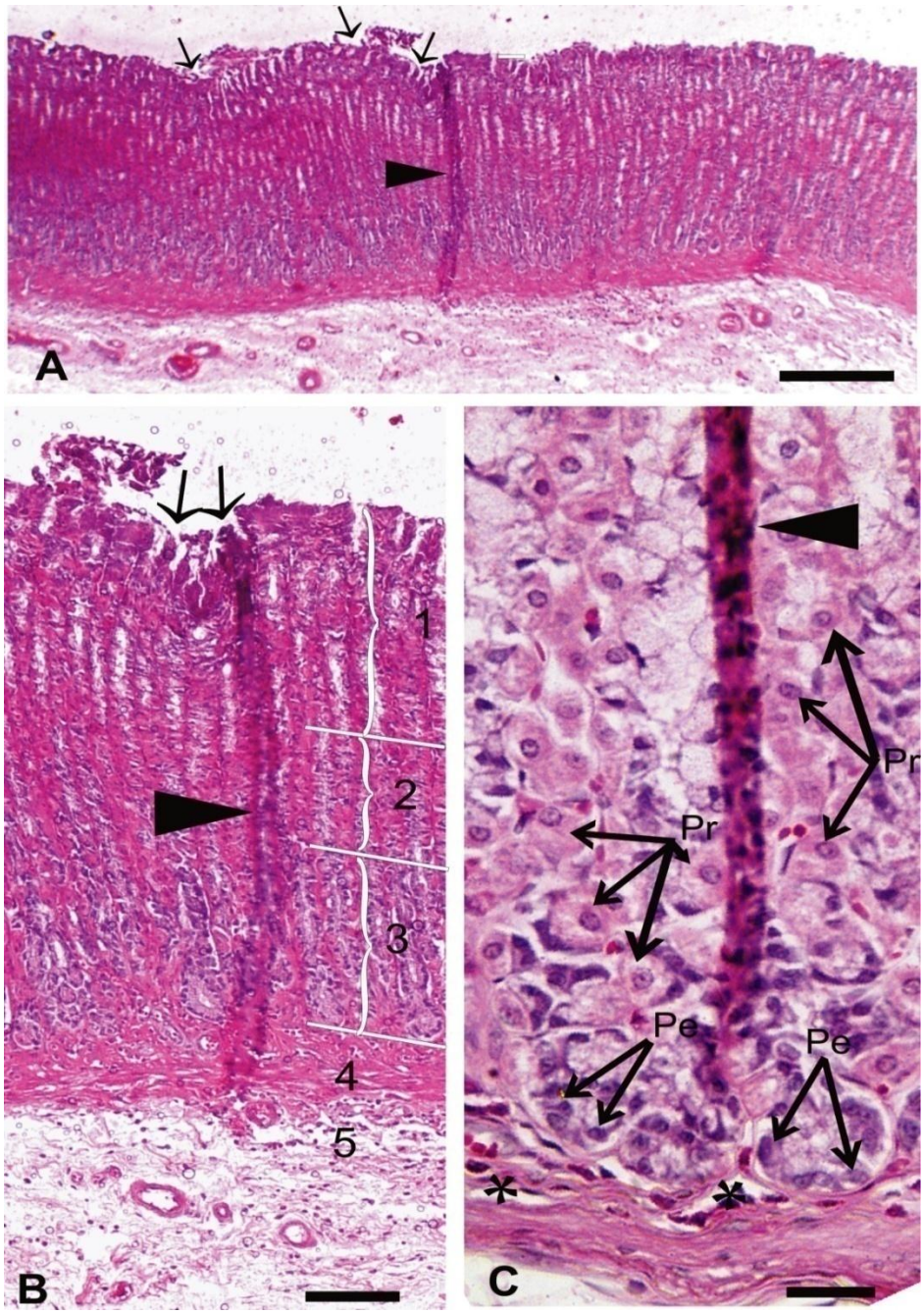


Figure 4-4: Histomicrographs showing the degree of ulceration in positive control rats (i.e. those that received distilled water before ulcer induction).

In A and B, areas of gastric mucosal surface erosion (arrows) occur at fairly short intervals. Penetrating ulcers (arrow heads) enter through the gastric pits (1), neck (2) and fundus/ body (3) of the gastric glands, muscularis mucosa (4) and to slightly extend, the tunica submucosa (5). More deeply, in C, the penetrating ulcer results in localized cell death involving peptic (Pe) and parietal cells (Pr) as well as degeneration (asterisks) in the muscularis mucosa. H&E, Bar in A, B & C= 300, 100 & 40μm respectively.

Light microscopy of the stomachs of the *S. nigrum* pretreated animals using H&E, Masson's trichrome stain and PAS revealed less degeneration of gastric gland cells and connective tissue, as well as less ulcer penetration into the gastric wall.

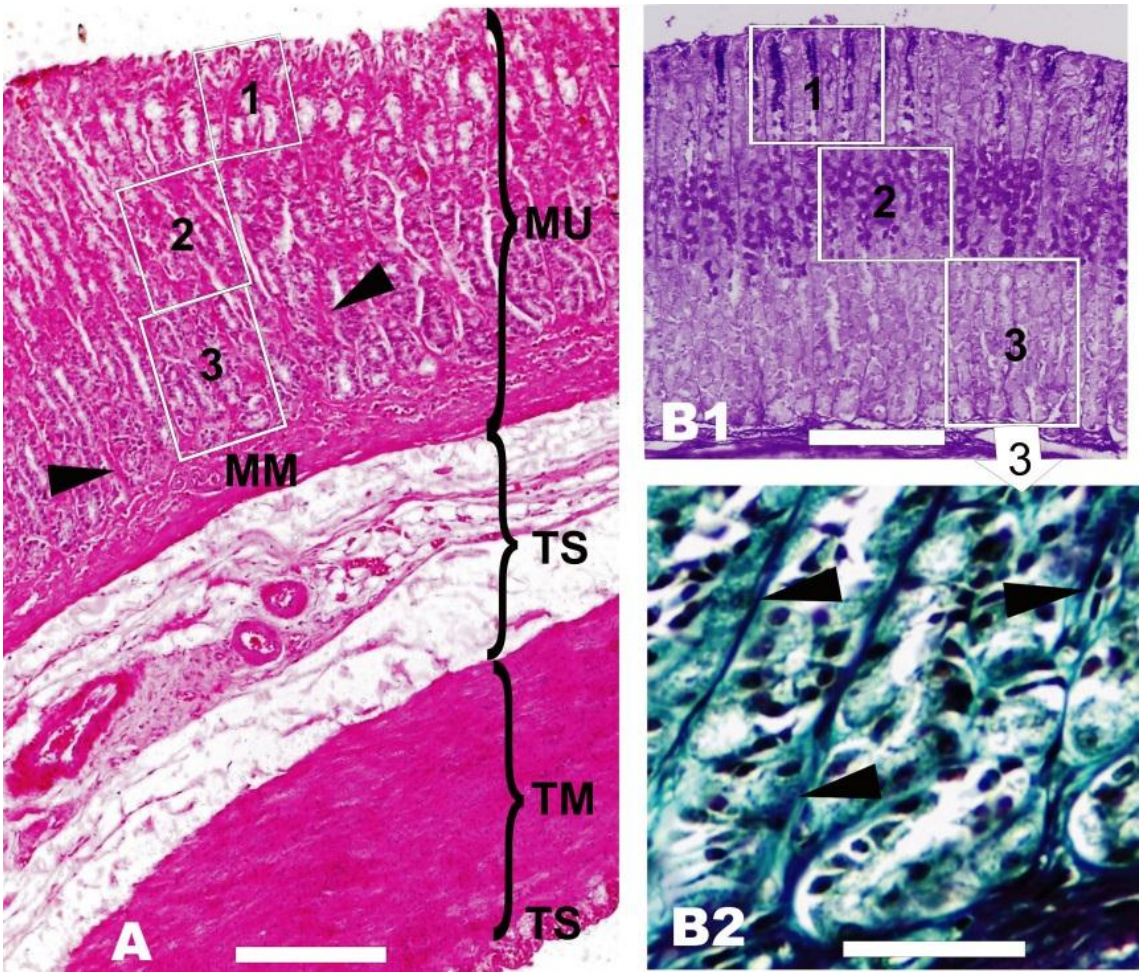


Figure 4-5: Microscopic view of the stomach wall in negative control rats.

A: Wall layers: tunica mucosa (MU) and its muscularis mucosa (MM), tunica submucosa (TS), tunica muscularis (TM) and tunica serosa (TS).

In A&B1: boxes show the regions of gastric gland pits (1), neck (2) and body (3). In B1, mucus-secreting cells of the gastric pits (1) stain more deeply with PAS as compared to those of the neck (2). Notice also (in A and more clearly in B2) the strands of muscularis mucosa (arrow heads) that extend apically to lie between the glands.

A-H&E, B1-PAS, B2- Masson's trichrome stain, Bar = 200μm in A & B and 50μm in C.

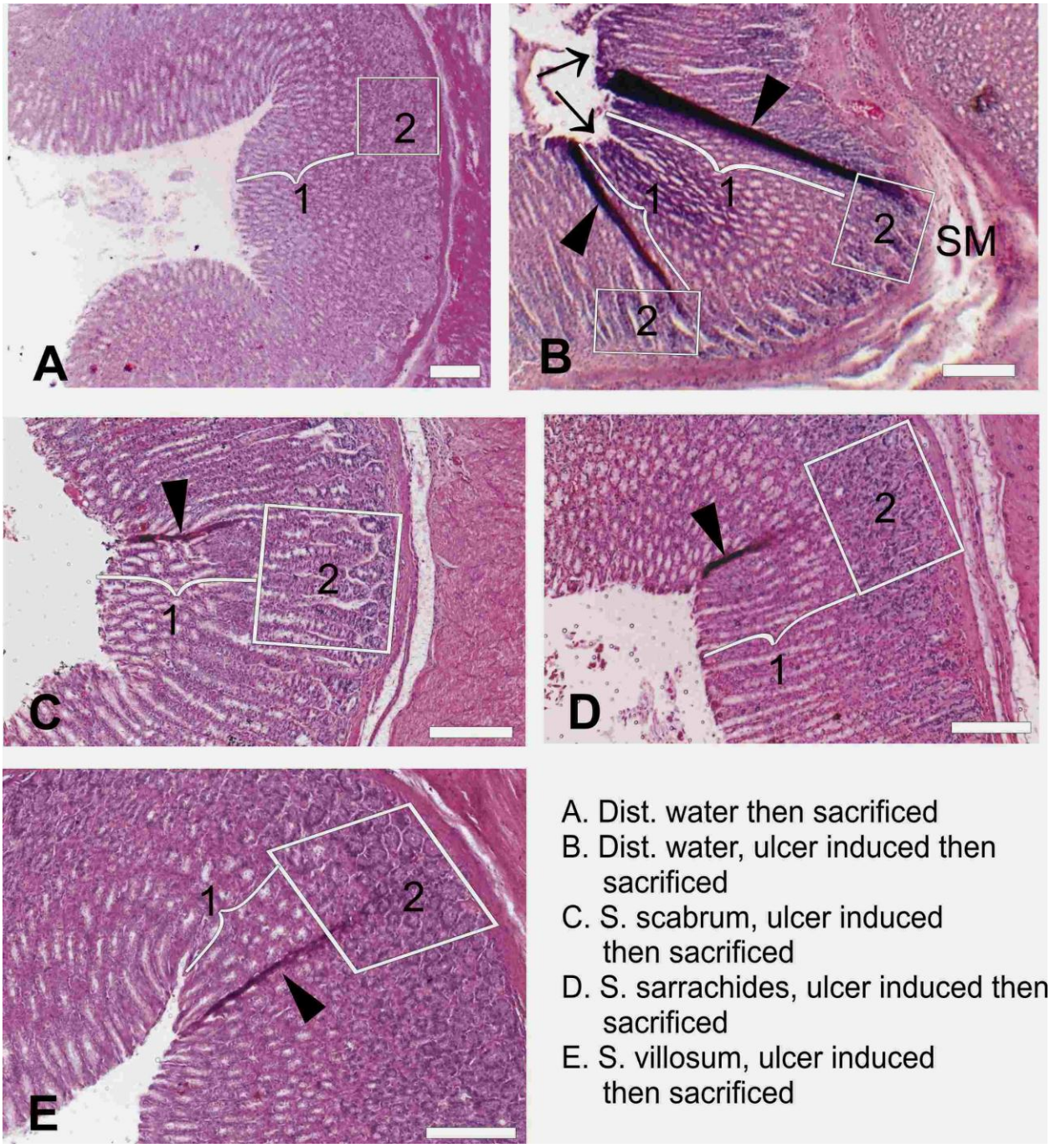


Figure 4.6: Histomicrographs showing the degree of ulceration in the five groups of rats.

Group A rats received only water before euthanasia and show no lesions. Rats in group B received water before ulcer induction. These present relatively more closely spaced ulcers (arrow heads) that begin as focal areas of erosion (arrows) on the mucosal surface and then penetrate deep through the gastric pits (1) and glands (2) to end at the tunica submucosa (SM). Groups C-E are the *S. scabrum*, *S. sarrachoides* and *S. villosum* - pretreated animals, respectively.

Mucosal surface erosion in these groups is relatively minimal and the penetrating ulcers only affect the gastric pits except in E where ulcers slightly extend to the upper gastric gland region. H&E, Bar = 200 μm in A-E.

Compared to negative controls (water only), PAS staining intensity of the mucus part of the gastric glands in *S. nigrum* pretreated animals did not reduce as much as it was seen in the positive control group (water then ethanol). Similarly, Masson's trichrome staining revealed thicker and more densely stained interglandular connective tissue separation in negative controls and pretreated *S. nigrum* treated rats as compared to positive controls (Table 4-2).

Table 4-2: Comparisons on intensity of staining of mucus part of the rat's gastric glands and of inter-glandular connective tissue in the different treatment groups

Treatment group	Negative control (water only)	Positive control (water then ethanol)	*Solanum scabrum* genotype	*Solanum sarrachoides* genotype	*Solanum villosum* genotype
PAS staining intensity	++++	+	+++	+++	++
Staining intensity for collagen	+++++	+	+++	+++	+++

4.4 Morphometric findings

4.4.1 Macroscopic ulcer index

Morphometric data from this study estimated the ulcerative index of the controls to be 13.46 ± 2.03mm, while for the *S. nigrum* - pretreated rats, the ulcer prevention index varied with the *S. nigrum* genotype as follows: 3.18, 3.70 and 4.94 in the *S. scabrum, S. sarrachoides* and *S. villosum* genotypes respectively. The percentage ulcer inhibition was 76.37, 72.51 and 63.30 in *S. scabrum, S. sarrachoides* and *S. villosum* respectively.

Data on the MaUI is shown in Table 4-3.

4.4.2 Microscopic ulcer index

The MiUI in the positive control animals was 14.5mm. This value decreased to 2.86, 4.08 and 6.12 in *S. scabrum, S. sarrachoides* and *S. villosum*, respectively, - in the pretreated rats. (Table 4-3)

Table 4-3: Protective effects of extracts from *S. scabrum, S. sarrachoides and S. villosum* genotypes of *S. nigrum* on ethanol induced gastric lesions in rats.

Treatment	Number of animals	Macroscopic ulcer index (mm)	Microscopic ulcer index (mm)	Inhibition Ratio (%)
+ve control- water then ulcer induction	6	13.46± 2.03	14.5± 2.17	
S. scabrum then ulcer induction	6	3.18± 0.64	2.86± 0.44	76.37
S. sarrachoides then ulcer induction	6	3.70± 0.73	4.08± 0.75	72.51
S. villosum then ulcer induction	6	4.94± 0.81	6.12± 0.83	63.30

CHAPTER FIVE

5 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Ethanol has been demonstrated to cause oxidative stress, leading to the release of Reactive Oxygen Species (ROS) in form of free radicals and consequently, damage to the gastric mucosa (Pan *et al*., 2008). The current work in rats shows that these lesions are restricted to the GP of the stomach and include edema, hyperemia, hemorrhage and necrotic bands (Hamid *et al*., 2012). The mechanism by which ethanol induces gastric ulcers entails a host of events. These include hyper production of free radicals, leading to increased lipid peroxidation and eventual damage of cells and their membranes (Shetty *et al*., 2000). In their study, Akhtar and Munir (1989) reported increased plasma concentrations of the hormone gastrin as well as increased gastric mucosal H+K+ATPase activity in ethanol induced gastric ulcerated rats.

S. *nigrum*, which is a locally available plant in many parts of the world, has been shown to prevent and in some cases treat gastric ulcers (Edmonds and Chweya, 1997; Jainu and Devi, 2004; Saravanan *et al*., 2011). *S. nigrum* has been reported to have both antisecretory and antioxidant activity thus inhibiting gastric ulcer formation in ethanol induced models (Sen *et al*., 2009). Previous studies have mostly used methanolic extracts of *S. nigrum* (Jainu and Devi, 2004: Sen *et al*., 2009: Saravanan *et al*., 2011). Studies have also shown that the phytochemical composition of plants differ with ecological zones where they are grown (Zouari *et al*., 2014; Yao and Bo, 2016).

The current study revealed that aqueous leaf extracts of three *S. nigrum* genotypes grown locally, protect against ethanol induced gastric ulcers, suggesting that these extracts have antioxidant and anti-inflammatory activities. The level of gastroprotection differed with the genotype. *S. scabrum* was the most effective at 76.37% ulcer inhibition compared to *S. sarrachoides* and *S. villosum* at 71.51% and 63.3% ulcer inhibition respectively. The macroscopic ulcer index was 13.46 in the control rats, decreasing to 3.18, 3.70 and 4.94 in the *S. scabrum, S. sarrachoides* and *S. villosum* genotypes respectively. Microscopic ulcer index scores decreased from 14.5mm in the positive control rats to 2.86, 4.08 and 6.12 in *S. scabrum, S. sarrachoides* and *S. villosum* -in the pretreated rats respectively, representing a 5.07, 3.55 and 2.37- fold decrease in the MiUI. These differences may be attributed to the variations in phytochemical composition of these genotypes. This is consistent with earlier studies where genetic differences have been shown to influence the growth and yield as well as the nature and composition of phytochemicals in medicinal plants (Ondieki *et al*, 2017:Sharma and Sarkar, 2013).

Results from this study compare favorably with earlier studies. The study by Saravanan *et al* (2011) analyzed for antiulcerogenic effects of methanolic extracts of Indian grown *S, nigrum* in aspirin and cold restraint stress induced ulcers, where ulcer inhibition rates were 77.85% and 66.67% respectively. In their study, Jainu and Devi (2004) tested the gastroprotective potential of the methanolic extracts of the berries of an Indian grown *S. nigrum* against aspirin induced ulcers in rats and documented ulcer inhibition scores at 49.30%, 70.12% and 72.70% at extract doses of 250, 500 and 1000mg/kg bwt respectively. The above studies reveal a dose dependent antiulcerogenic activity of S. *nigrum*.

A study by El-Meligy *et al*., (2016) where rats were pretreated with a methanolic extract of *S. nigrum* and ulcers induced using absolute alcohol showed dose dependent reduction of ulcer index, with doses of 125, 250 and 500mg/kg bwt resulting in ulcer index of 8.30, 5.00 and 2.80 respectively. This is consistent with the current preliminary study where a dose comparison between 300 and 500mg/kg bwt resulted in reduced ulceration at the latter dose. The preferred dose rate of 500mg/kg bwt in the rat, corresponds to that of 35g per 70kg person (WHO, 1993).

Herbal medicines are increasingly being tested for their potential as safer and more readily available antiulcer remedies. The current acute toxicity study revealed no adverse effects of the three extracts at a dose of 2000 mg/kg bwt. Herbal medicines mainly act by increasing mucus production as well as decreasing gastric acid production (Luiz-Ferreira *et al*., 2010). Their activity is attributed to their phytochemical composition. Tannins, terpenes, flavonoids, ascorbic acid and fatty acids are reported in several studies (Alkofahi and Atta, 1999; Hiruma-Lima *et al*., 2001; Alese *et al*., 2018) as key phytoconstituents of plants showing gastroprotective activity.

In the current study, *S. nigrum* extracts tested positive for tannins, saponins, terpenoids and flavonoids. Pretreatment of rats with these extracts was further shown to inhibit ethanol-induced gastric injuries that include edema, congestion, hemorrhage and necrosis. Tannins have been shown to have multiple benefits in disease management. They are considered as anti-inflammatory, antioxidant, hemostatic, antiseptic as well as an analgesic (De Jesus *et al*., 2012). They are found in most plants having antiulcerogenic properties, where they were reported to be involved in the anti-inflammatory process, to promote tissue repair and to have activity against *H.*

pylori (Falcão *et al*., 2008). They promote healing by forming a protective tannin-polysaccharide complex layer over injured epithelium, allowing healing to take place beneath the layer (De Jesus *et al*., 2012).

Saponins have a potent gastroprotective effect. A study by Yoshikawa *et al* (2005) found that saponins inhibited both ethanol and indomethacin-induced gastric ulcers in rats. They further isolated the saponins and found several saponin types to have gastric ulcer inhibitory activity at a dose of 5mg/kg bwt, with their activity being stronger than that of omeprazole.

Flavonoids are a diverse class of secondary metabolites having both ulcer prevention and ulcer healing potential (La Casa *et al*., 2000; de Lira Mota *et al*., 2009). They have been shown to have antioxidant activity (Hanasaki *et al*., 1994). A flavonoid rich fraction of a methanolic extract of the *M. paradisiaca* fruit, was found to progressively restore damaged epithelium in aspirin induced gastric lesions in the rat (Alese *et al*., 2018).

Terpenes play a vital role in growth and development of plants as well as defense against environmental stress (Mewalal *et al*., 2017). They possess a rich variety of biological activities such as anti- neoplastic, anti-inflammatory and anti-microbial effects (Rufino *et al*., 2014). Terpenes possess antiulcer activity with various terpenoids being reported to protect against different ulcerogens, with most showing dose dependent activity (Pertino *et al*., 2006: Pertino *et al*., 2007).

Periodic acid–Schiff (PAS) is a staining method used to detect polysaccharides such as glycogen, mucosubstances such as glycoproteins, glycolipids and mucins in tissues (Burkitt *et al*., 1993). In the staining, the reaction of periodic acid results in oxidization of vicinal diols and creation of aldehydes which then react with the Schiff reagent to give a purple-magenta color (Ishihara *et al*., 1996; Karasawa *et al*., 2012). In the stomach, gastric mucins occur as surface mucin and gland mucin, both of which are best demonstrated by the PAS (Ota *et al*., 1991). Findings from this study indicate that ethanol-induced gastric ulceration is associated with a decrease in mucin and number of mucus secreting cells as revealed by PAS stained sections where sections from positive control rats showed a decreased intensity of staining. It appears therefore that *S. nigrum* attenuated these effects resulting in increased PAS staining intensity in the pretreated rats.

Earlier workers (Slomiany *et al*., 1997) have shown that gastric ulceration involves a decrease in synthesis of prostaglandins which results in reduction in mucous secretion and direct damage to the mucin layer or mucin synthesis. Indeed, mucin acts as a primary barrier to acid-induced damage in the stomach (Lichtenberger, 1995) by acting as a free radical scavenger due to its ability to bind to lipids (Prathama and Harendra-Kumar, 2012).

Masson's trichrome is a three-colour staining protocol used in histology to distinguish cells from surrounding connective tissue, with most recipes coloring collagen blue or green, cytoplasm light red or pink and nuclei dark brown to black (Burkitt *et al*., 1993). Studies have shown that tissues stained with Masson's trichrome present a clearer view of collagen fiber deposition and reorganization compared with H&E thereby giving a more accurate observation and analysis of changes in tissue structure (Rieppo *et al*., 2019; Suvik & Effendy, 2012). In this study, Masson's Trichrome stained sections showed reduced intensity of collagen staining in the rats that were not

pretreated with any extract before ulcer induction compared to the negative control animals and those pretreated with *S. nigrum*. This may be due to dense inflammation of gastric mucosa which caused destruction of the gastric glands and connective tissue (Alese *et al*., 2018).

Ulcer depth also decreased in rats pretreated with the *S. nigrum* extracts where the ulcers in these treatment groups did not penetrate beyond the area of the gastric pits, except for the S. villosum where the ulcers penetrated into the gastric glands. These however did not reach the tunica submucosa as was the case in the positive control animals indicating that pretreatment with *S. nigrum* lowered the magnitude of damage induced on the gastric mucosa by ethanol.

The integrity of gastric mucosal cells, particularly parietal and chief cells, is usually affected by exogenous and endogenous stimuli-induced gastritis (Kengkoom *et al*., 2017). In this study, peptic and parietal cell death was evident in positive controls but not in *S. nigrum*-pretreated rats implying that *S. nigrum* conferred substantial degree of cytoprotection against damage by ethanol. This appears to be in tandem with the findings of Kengkoom *et al*., (2017) in which omeprazole (an allopathic drug that also treats gastric ulcers by blocking acid secretion) protected rats against injury induced on peptic and parietal cells by ethanol.

5.2 Conclusion

In conclusion, this study serves to demonstrate that aqueous. leaf extracts of *S. scabrum, S. sarrachoides* and *S. villosum* genotypes of *S. nigrum* Linn grown in Kenya seem to reduce the risk of gastric ulceration. It also shows genotype dependent differences in ulcer index scores

where an index of 3.18, 3.70 and 4.9 was demonstrated in the *S. scabrum, S. sarrachoides* and *S. villosum* genotypes respectively. The percentage ulcer inhibition was 76.37, 72.51 and 63.30 in *S. scabrum, S. sarrachoides* and *S. villosum* respectively. These differences may be attributed to variations in phytochemical composition of these genotypes.

5.3 Recommendations

The following recommendations can be made from the results of this study

1. Policies to foster increased awareness, cultivation and consumption of *S. scabrum*, *S. sarrachoides* and *S. villosum* for their demonstrated potential to prevent gastric ulcers as well as other illnesses cited in the literature.
2. Further isolation, quantification and characterization of phytochemicals responsible for the antiulcerogenic potential of these three genotypes in the *S. nigrum* complex for purposes of commercial exploitation.
3. Studies to determine the best maturity stage at which to harvest *S. nigrum* in order to have the highest concentration of phytochemicals responsible for antiulcerogenic activity.
4. Further studies to determine or rule out potentially toxic effects of the three *S. nigrum* genotypes' extracts should be carried out to validate their use as alternative antiulcerogenic compounds.
5. Further studies to determine the mechanism by which the three *S. nigrum* genotype extracts exact antiulcerogenic activity should be carried out.
6. A dose dependent study of the *S. nigrum* extracts should be carried out to determine the most effective dose of the extracts.

REFERENCES

Akhtar, M. S., & Munir, M. (1989). Evaluation of the gastric antiulcerogenic effects of Solanum nigrum, Brassica oleracea and Ocimum basilicum in rats. Journal of ethnopharmacology, **27(1-2)**, 163-176.

Alese, M. O., Adewole, O. S., Ijomone, O. M., Ofusori, D. A., &Alese, O. O. (2018). Mucus secretion and collagen fibres integrity are compromised in aspirin induced gastric lesion; protective role of Musa paradisiaca. Italian Journal of Anatomy and Embryology, **123(2),** 136-148.

Alkofahi, A., & Atta, A. H. (1999). Pharmacological screening of the anti-ulcerogenic effects of some Jordanian medicinal plants in rats. Journal of Ethnopharmacology, **67(3)**, 341-345.

Atherton, J. C. (2006). The pathogenesis of Helicobacter pylori-induced gastro-duodenal diseases. *Annual review of pathology*, ***1***, 63-96.

Awaad, A. S., El-Meligy, R. M., & Soliman, G. A. (2013). Natural products in treatment of ulcerative colitis and peptic ulcer. *Journal of Saudi chemical society*, ***17*****(1)**, 101-124.

Bhattacharjee, M., Bhattacharjee, S., Gupta, A., & Banerjee, R. K. (2002). Critical role of an endogenous gastric peroxidase in controlling oxidative damage in H. pylori-mediated and nonmediated gastric ulcer. *Free Radical Biology and Medicine*, ***32*****(8)**, 731-743.

Bhattacharyya, A., Chattopadhyay, R., Mitra, S., & Crowe, S. E. (2014). Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev*, ***94***, 329-354.

Bighetti, A. E., Antonio, M. A., Kohn, L. K., Rehder, V. L. G., Foglio, M. A., Possenti, A., ... &Carvalho, J. E. (2005). Antiulcerogenic activity of a crude hydroalcoholic extract and coumarin isolated from *Mikania laevigata* Schultz Bip. *Phytomedicine*, ***12*****(1)**, 72-77.

Bodeker, C., Bodeker, G., Ong, C.K., Grundy, C.K., Burford, G., & Shein, K. (2005). WHO global atlas of traditional, complementary and alternative medicine. Geneva, Switzerland: World Health Organization.

Brzozowski, T., Konturek, P. C., Konturek, S. J., Pajdo, R., Kwiecien, S., Pawlik, M., ... & Pawlik, W. W. (2004). Ischemic preconditioning of remote organs attenuates gastric ischemia–reperfusion injury through involvement of prostaglandins and sensory nerves. *European journal of pharmacology*, ***499*****(1-2)**, 201-213.

Burkitt, H.G., Young, B., Heath, J.W., 1993. Wheater's Functional Histology: A Textbook and Color Atlas, third ed. Churchill Livingstone, Edinburgh

Carleton, H. M., Drury, R. A. B., & Wallington, E. A. (1980).*Carleton's histological technique*. Oxford University Press, USA.

Chivers, D. J., & Hladik, C. M. (1980). Morphology of the gastrointestinal tract in primates: comparisons with other mammals in relation to diet. Journal of morphology, **166(3)**, 337-386.

Colville, T. P., &Bassert, J. M. (2015). Clinical anatomy and physiology for veterinary technicians. Elsevier Health Sciences.pp392-402

Colville, T. P., & Bassert, J. M. (2016). Clinical anatomy and physiology for veterinary technicians, **3rd Edn**. St. Louis, Missouri: Elsevier.

Correa, P., & Houghton, J. (2007). Carcinogenesis of Helicobacter pylori. *Gastroenterology*, ***133*(2)**, 659-672.

De Jesus, N. Z. T., Falcão, H. D. S., Gomes, I. F., Leite, T. J. D. A., Lima, G. R. D. M., Barbosa-Filho, J. M., ... & Batista, L. M. (2012). Tannins, peptic ulcers and related mechanisms. *International journal of molecular sciences*, ***13*(3)**, 3203-3228.

de Lira Mota, K. S., Dias, G. E. N., Pinto, M. E. F., Luiz-Ferreira, Â., Monteiro Souza-Brito, A. R., Hiruma-Lima, C. A., ... & Batista, L. M. (2009). Flavonoids with gastroprotective activity.Molecules, **14(3)**, 979-1012.

de Souza Almeida, E. S., CechinelFilho, V., Niero, R., Clasen, B. K., Balogun, S. O., & de Oliveira Martins, D. T. (2011). Pharmacological mechanisms underlying the anti-ulcer activity of methanol extract and canthin-6-one of Simaba ferruginea A. St-Hil. in animal models. *Journal of ethnopharmacology*, ***134*(3),** 630-636.

Ding, S. Z., Goldberg, J. B., & Hatakeyama, M. (2010). Helicobacter pylori infection, oncogenic pathways and epigenetic mechanisms in gastric carcinogenesis. *Future oncology*, ***6*(5)**, 851-862.

Eberle, J. A. M., Widmayer, P., &Breer, H. (2014). Receptors for short-chain fatty acids in brush cells at the "gastric groove". *Frontiers in physiology*, ***5***, 152.

Edmonds, J. M., &Chweya, J. A. (1997).*Black nightshades: Solanum nigrum L. and related species* (**Vol. 15**). Bioversity International.

El-Meligy, R. M., Awaad, A. S., Soliman, G. A., Kenawy, S. A., &Alqasoumi, S. I. (2017). Prophylactic and curative anti-ulcerogenic activity and the possible mechanisms of action of some desert plants. *Saudi Pharmaceutical Journal*, ***25*(3)**, 387-396.

Ernst, E., & Hung, S.K. (2011). Great expectations: what do patients using complementary and alternative medicine hope for? Patient, **4**, 89-101.

Eurell, J. A., & Frappier, B. L. (Eds.). (2013). *Dellmann's textbook of veterinary histology*. John Wiley & Sons.

Falcão, H. S., Mariath, I. R., Diniz, M. F. F. M., Batista, L. M., & Barbosa-Filho, J. M. (2008). Plants of the American continent with antiulcer activity. *Phytomedicine*, ***15*(1-2)**, 132-146.

FAO, 2006. World base reference for soil resources. Report on World Soil Resources. FAO. Rome, Italy.

Flemström, G., & Turnberg, L. A. (1984). Gastroduodenal defence mechanisms. Clinics in gastroenterology, **13(2),** 327-354.

Ghoshal, N. G., &Bal, H. S. (1989). Comparative morphology of the stomach of some laboratory mammals. *Laboratory animals*, ***23*(1)**, 21-29.

Greaves, P. (2012). *Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation*. **4th ed**. Cambridge,Academic Press.

Grover, J. K., Adiga, G., Vats, V., &Rathi, S. S. (2001). Extracts of Benincasahispida prevent development of experimental ulcers. *Journal of Ethnopharmacology*, ***78*(2-3)**, 159-164.

Guzmán-Gómez, O., García-Rodríguez, R., Quevedo-Corona, L., Pérez-Pastén-Borja, R., Rivero-Ramírez, N., Ríos-Castro, E., ...& Chamorro-Cevallos, G. (2018). Amelioration of Ethanol-Induced Gastric Ulcers in Rats Pretreated with Phycobiliproteins of Arthrospira (Spirulina) Maxima. *Nutrients*, ***10*****(6)**, 763.

Hamid, R. A., Foong, C. P., Ahmad, Z., & Hussain, M. K. (2012). Antinociceptive and anti-ulcerogenic activities of the ethanolic extract of Annona muricata leaf. *Revista Brasileira de Farmacognosia*, ***22*****(3)**, 630-641.

Hanasaki, Y., Ogawa, S., & Fukui, S. (1994). The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Radical Biology and Medicine*, ***16*****(6),** 845-850.

Hiruma-Lima, C. A., Gracioso, J. S., Toma, W., Almeida, A. B., Paula, A. C., Brasil, D. S. B., ... & Brito, A. S. (2001). Gastroprotective effect of aparisthman, a diterpene isolated from Aparisthmium cordatum, on experimental gastric ulcer models in rats and mice. Phytomedicine, **8(2)**, 94-100.

Huang, X. R., Hui, C. W. C., Chen, Y. X., Chun, B., Wong, Y., Fung, P. C., ... & Lam, S. K. (2001). Macrophage migration inhibitory factor is an important mediator in the pathogenesis of gastric inflammation in rats. *Gastroenterology*, ***121,*** 619-630.

Jaarin, K., Gapor, M. T., Nafeeza, M. I., &Fauzee, A. M. (2002). Effect of various doses of palm vitamin E and tocopherol on aspirin- induced gastric lesions in rats.*International journal of experimental pathology*, ***83*****(6)**, 295-302.

Ishihara, K., Kurihara, M., Goso, Y., Urata, T., Ota, H., Katsuyama, T., & Hotta, K. (1996). Peripheral α-linked N-acetylglucosamine on the carbohydrate moiety of mucin derived

from mammalian gastric gland mucous cells: epitope recognized by a newly characterized monoclonal antibody. *Biochemical Journal*, ***318*****(2)**, 409-416.

Jainu, M., & Devi, C. S. (2004). Antioxidant effect of methanolic extract of *Solanumnigrum* berries on aspirin induced gastric mucosal injury. *Indian Journal of Clinical Biochemistry*, ***19*****(1)**, 57-61.

Jainu M., Mohan V., Devi S. (2006) Protective effect of *Cissus quadrangularis* on neutrophil mediated tissue injury induced by aspirin in rats. J. Ethnopharmacol. **104**: 302-305.

Jagatheeswari, D., Bharathi, T., & Ali, H. S. J. (2013). Black Night Shade (Solanum nigrum L.)-An Updated Overview. *Int. J. Pharm. Biol. Arch*, ***4***, 288-295.

Jian, L. I. (2008). Study on Antitumor Activity and Immunomodulating Effect of Polysaccharides Isolated from *SolanumnigrumLinne* [J]. *Journal of Anhui Agricultural Sciences*, ***33***, 100.

Kadhin G, Omar H, Ismail A (2015). Risk factors associated with peptic ulcer disease.J.Bioengineering and Biomedical Sci.**5**:142 doi.10.4172/2155-9538.1000142

Kang, J. Y., Teng, C. H., & Chen, F. C. (1996). Effect of capsaicin and cimetidine on the healing of acetic acid induced gastric ulceration in the rat. *Gut*, ***38*****(6)**, 832-836.

Karasawa, F., Shiota, A., Goso, Y., Kobayashi, M., Sato, Y., Masumoto, J., ... & Ueda, M. (2012). Essential role of gastric gland mucin in preventing gastric cancer in mice. *The Journal of clinical investigation*, ***122*****(3)**, 923-934.

Kavoi B, Makanya A, Hassanali J, Carlsson H, Kiama S. 2010. Comparative functional structure of the olfactory mucosa in the domestic dog and sheep. Ann Anat **192**:329–337

Kengkoom, K., Tirawanchai, N., Angkhasirisap, W., & Ampawong, S. (2017). Omeprazole preserves the RER in chief cells and enhances re-epithelialization of parietal cells with SOD and AQP-4 up-regulation in ethanol-induced gastritis rats. *Experimental and therapeutic medicine*, ***14*****(6)**, 5871-5880.

Khazaei, M., &Salehi, H. (2006). Protective effect of *Falcaria vulgaris* extract on ethanol induced gastric ulcer in rat. *Iranian Journal of Pharmacology & Therapeutics*, ***5*****(1)**, 43-46.

Knill-Jones, R., Drummond, M., Kohli, H., & Davies, L. (1990). Economic evaluation of gastric ulcer prophylaxis in patients with arthritis receiving non-steroidal anti-inflammatory drugs. *Postgraduate Medical Journal*, ***66*****(778)**, 639-646.

Kołodziejska, J., & Kołodziejczyk, M. (2018). Diclofenac in the treatment of pain in patients with rheumatic diseases. Reumatologia, **56**,174-183.

Kondo, Y. (1966). The functional morphological studies on the fundic glands i. Histological study with special reference to the length, branching, and parenchymal cell ratios of the glands. Tohoku journal of agricultural research, **17(1),** 65-79

Konturek, S. J. (1990). Mechanisms of gastroprotection. Scandinavian Journal of Gastroenterology, **25(sup174),** 15-28.

Koolhaas, J. M. (1999). The laboratory rat.In The UFAW Handbook on the Care and Management of Laboratory Animals, **7th ed**. (T. Poole, Ed.), Vol. 1, pp. 313–330. Blackwell Science, Oxford, UK

La Casa, C., Villegas, I., De La Lastra, C. A., Motilva, V., & Calero, M. M. (2000). Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. *Journal of ethnopharmacology*, ***71*****(1-2)**, 45-53.

Lahlou, M. (2013). The Success of Natural Products in Drug Discovery. Pharmacol. Pharmacy **4**: 17-31.

Lichtenberger, L. M., Wang, Z. M., Romero, J. J., Ulloa, C., Perez, J. C., Giraud, M. N., & Barreto, J. C. (1995). Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. *Nature medicine*, ***1*****(2)**, 154-158.

LoIudice, T. A., Saleem, T., & Lang, J. A. (1981).Cimetidine in the treatment of gastric ulcer induced by steroidal and nonsteroidal anti-inflammatory agents. *American Journal of Gastroenterology*, ***75*****(2).**

Luciano, L., &Reale, E. (1992). The "limiting ridge" of the rat stomach. *Archives of histology and cytology*, ***55***(Supplement), 131-138.

Luiz-Ferreira, A., Almeida, A. C. A. D., Cola, M., Barbastefano, V., Almeida, A. B. A. D., Batista, L. M., ... & Vilegas, W. (2010). Mechanisms of the gastric antiulcerogenic activity of Anacardium humile St. Hil on ethanol-induced acute gastric mucosal injury in rats. *Molecules*, ***15*****(10)**, 7153-7166.

Majeed, W., Khaliq, T., Aslam, B., Khan, J. A., & Iftikhar, A. (2015). Medicinal plants with gastroprotective potential. *Bangladesh Journal of Pharmacology*, ***10*****(3),** 588-603.

Maobe M, Gatebe E, Gitu L, Rotich H (2013).Preliminary phytochemical screening of Eight selected medicinal herbs used for treatment of diabetes,malaria and pneumonia in

KisiiRegion.South Kenya. European Journal of AplliedScciences**5 (1)**:10.5829/idosi.ejas.2013.5.16512

Marcus, G.M., Smith, L.M., Scheinman, M.M., Badhwar, N., Lee, R.J., Tseng, Z.H., Lee, B.K, Kim, A., & Olgin, J.E. (2010). Proton pump inhibitors are associated with focal arrhythmias. The Journal of Innovations in Cardiac Rhythm Management,**1**, 85-89.

Martin S.W., Meek A.H., Willeberg P. (1987): Veterinary Epidemiology. Principles and Methods. Iowa State University Press, Ames, p. 32.

Matasyoh G and Mwaura H (2014). The *Solanumnigrum*complex in western Kenya.Journal of plant,animal and environmental sciences.ISSN 2231 4490

Mewalal, R., Rai, D. K., Kainer, D., Chen, F., Külheim, C., Peter, G. F., & Tuskan, G. A. (2017). Plant-derived terpenes: a feedstock for specialty biofuels. *Trends in biotechnology*, ***35*(3)**, 227-240.

Miaron, O.J., Kassim O.F., Wallington E.N. (2004). Indigenous knowledge: The basis of the maasaiEthnoveterinary diagnostic skills. Journal of Human Ecology **16 (1):**43-48

Milosavljevic I, Kostic M, Jovanovic I (2011). Complication of peptic ulcer disease. J digestive diseases **29**:491-493 doi 10.1159/000331517

Mishra, S. P., & Singh, S. (2012). Processing of Moringa Oleifera leaves for human consumption. Bulletin of Environment, Pharmacology and life sciences. **2(1)**. 28-31

Mizui, T., &Doteuchi, M. (1983). Effect of polyamines on acidified ethanol-induced gastric lesions in rats.*The Japanese Journal of Pharmacology*, ***33*(5)**, 939-945.

Moore, R. A., Derry, S., Simon, L. S., & Emery, P. (2014). Nonsteroidal Anti- Inflammatory Drugs, Gastroprotection, and Benefit–Risk. *Pain Practice*, ***14*(4)**, 378-395.

Mosihuzzaman, M (2012) . Herbal medicines in healthcare an overview. Natural products communication 2012 ;**7 (6**).807-809

Najm, W. I. (2011). Peptic ulcer disease.*Primary Care: Clinics in Office Practice*, **38(3)**, 383-394.

Nanyingi M, Mbaria J, Lanyasuny A, Wagate C, Koros K, Kaburia H, Wagate R, Ogara W (2008).Ethnopharmacological survey of Samburu Distric, Kenya.Journal of Ethnobiology and Ethnomedicine 4:4-14

Odou, P., Martin, P., Membré, S., Gressier, B., Tamiji, L., Dine, T., ...&Moulron, S. (1999). Omeprazole- induced leukopenia.A case report.*Journal of clinical pharmacy and therapeutics*, ***24*(5)**, 317-321.

OECD 2000. Guidance document on acute oral toxicity 423.Enviromental Health and Safety monograph series on testing assessment 2000;No.24.

Ondieki, G., Nyagblordzro, M., Kikete, S., Liang, R., Wang, L., & He, X. (2017). Cytochrome P450 and P-glycoprotein-mediated interactions involving African herbs indicated for common noncommunicable diseases. Evidence-Based Complementary and Alternative Medicine, 2017.

Ontita E.G., Onyango C.M., Onwonga R.N. and Nyamongo D. (2016).Indigenous Knowledge on the Uses of African Nightshades (*Solanum nigram* L.) Species among Three Kenyan Communities. *Asian Journal of Agricultural Extension, Economics & Sociology. **14(3)**: 1-8*

Onyango, A. M. O. (2010). African indigenous vegetables in Kenya: Strategic repositioning in the horticultural sector. Nairobi: Jomo Kenyatta University of Agriculture and Technology.

Orodho, A. B. (2006). Country Pasture/Forage Resource Profiles: Kenya. *FAO (Food and Agriculture Organization) http://www.fao. org/ag/AGP/AGPC/doc/Counprof/Kenya. htm Accessed*, *27*.

Ota, H., Katsuyama, T., Ishii, K., Nakayama, J., Shiozawa, T., & Tsukahara, Y. (1991). A dual staining method for identifying mucins of different gastric epithelial mucous cells. *The Histochemical journal*, ***23*(1)**, 22-28.

Pan, J. S., He, S. Z., Xu, H. Z., Zhan, X. J., Yang, X. N., Xiao, H. M., ... & Ren, J. L. (2008). Oxidative stress disturbs energy metabolism of mitochondria in ethanol-induced gastric mucosa injury. World journal of gastroenterology: WJG, **14(38)**, 5857.

Pertino M, Rodríguez JA, Theoduloz C, Razmilic I, Schmeda-Hirschmann G. (2006) Gastroprotective activity and cytotoxic effect of cyperenoic acid derivatives. Journal of Pharmacy and Pharmacology, **58**, 1507-1513.

Pertino, M., Schmeda-Hirschmann, G., Rodríguez, J. A., & Theoduloz, C. (2007). Gastroprotective effect and cytotoxicity of terpenes from the Paraguayan crude drug "yagua rova"(Jatropha isabelli). *Journal of Ethnopharmacology*, ***111*(3)**, 553-559.

Potawale, S. E., Sinha, S. D., Shroff, K. K., Dhalawat, H. J., Boraste, S. S., Gandhi, S. P., & Tondare, A. D. (2008). Solanum nigrum Linn.: A phytopharmacological review. *Pharacologyonline*, ***3***, 140-163.

Pour, B. M., Latha, L. Y., & Sasidharan, S. (2011). Cytotoxicity and Oral Acute Toxicity Studies of Lantana camara Leaf Extract. *Molecules*, ***16,*** 3663-3674.

Pandian, R. S., Anuradha, C. V., &Viswanathan, P. (2002). Gastroprotective effect of fenugreek seeds (Trigonellafoenumgraecum) on experimental gastric ulcer in rats. *Journal of ethnopharmacology*, ***81*****(3)**, 393-397.

Peskar, B. M. (2001). Neural aspects of prostaglandin involvement in gastric mucosal defense. *J PhysiolPharmacol*, ***52*****(4)**, 555-568.

Pilotto, A., Franceschi, M., Leandro, G., Paris, F., Niro, V., Longo, M. G., ...& Di Mario, F. (2003). The risk of upper gastrointestinal bleeding in elderly users of aspirin and other non-steroidal antiinflammatory drugs: the role of gastroprotective drugs. *Aging clinical and experimental research*, ***15*****(6)**, 494-499.

Pichop, G. N., & Weinberger, K. (2009). Marketing of African indigenous vegetables along urban and peri-urban supply chains in sub-Saharan Africa.In *African indigenous vegetables in urban agriculture* (pp. 257-276).Routledge.

Prathima, S., & Kumar, H. M. L. (2012). Mucin profile of upper gastrointestinal tract lesions. *J Clin Biomed Sci*, ***2*****(4)**, 185-191.

Ranakrishnan K and Salina R C (2007). Peptic ulcer desease.AFam physician 1:**7 (7)** :1005-12

Rawani, A., Ghosh, A., & Chandra, G. (2013). Mosquito larvicidal and antimicrobial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of Solanumnigrum L.(Solanaceae: Solanales). *Actatropica*, *128*(3), 613-622.

Rawani, A., Ghosh, A., & Chandra, G. (2013). Mosquito larvicidal and antimicrobial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of Solanum nigrum L.(Solanaceae: Solanales). Acta Tropica, **128(3)**, 613-622.

Rieppo L, Janssen L, Rahunen K, Lehenkari P, Finnilä MAJ, Saarakkala S (2019) Histochemical quantification of collagen content in articular cartilage. PLoS ONE **14(11)**:

Robert, A. (1979). Cytoprotection by prostaglandins. Gastroenterology, **77(4 Pt 1)**, 761-767.

Romero-Gomez, M., Otero, M. A., Suarez-Garcia, E., Diaz, E. G., Fobelo, M. J., & Castro-Fernandez, M. (1999). Acute hepatitis related to omeprazole. *The American journal of gastroenterology*, ***94*(4),** 1119-1120.

Rufino, A. T., Ribeiro, M., Judas, F., Salgueiro, L., Lopes, M. C., Cavaleiro, C., & Mendes, A. F. (2014). Anti-inflammatory and chondroprotective activity of (+)-α-pinene: structural and enantiomeric selectivity. *Journal of natural products*, ***77*(2)**, 264-269.

Sabiu, S., Garuba, T., Sunmonu, T., Ajani, E., Sulyman, A., Nurain, I., &Balogun, A. (2015). Indomethacin-induced gastric ulceration in rats: Protective roles of *Spondiasmombin* and *Ficusexasperata*. *Toxicology Reports*, ***2***, 261-267.

Sannomiya, M., Fonseca, V. B., Da Silva, M. A., Rocha, L. R. M., Dos Santos, L. C., Hiruma-Lima, C. A., ... &Vilegas, W. (2005). Flavonoids and antiulcerogenic activity from Byrsonimacrassa leaves extracts. *Journal of Ethnopharmacology*, ***97*(1)**, 1-6.

Saravanan S, Dhasarathan P, Indira V, Venkatraman R (2011). Gastro protective and antioxidant Activity of *Solanumnigrum Linn*. againstAsprin and Cold Restraint Stress induced Ulcerated rats.Research Journal of immunology ISSN 1994-7909/DOI:10.3923/rji.2011

Schippers R.R. 2000. African Indigenous Vegetables.An Overview of the Cultivated Species.Natural Resources Institute, Chatham, UK.

Segottaiyan A, Praburam L, Manoharan K (2012).Hypoglycaemic and hypolipidemic activity of *Solanumnigrumin*alloxan induced diabetic albino rats.IntJ.pharmSciRes :Vol**3 (8)** 2841-2848

Sen, S., Chakraborty, R., De, B., &Mazumder, J. (2009). Plants and phytochemicals for peptic ulcer: An overview. Pharmacognosy Reviews, **3(6)**, 270.

Sharma, V., &Sarkar, I. N. (2013).Leveraging biodiversity knowledge for potential phyto-therapeutic applications. Journal of the American Medical Informatics Association, **20(4),** 668-679.

Shetty, R., Kumar, V., Naidu, M. U. R., &Ratnakumar, K. S. (2000). Effect of Ginkgo biloba extract in ethanol induced gastric mucosal lesions in rats. *Indian J. Pharmacol*, ***32***, 313-317.

Slomiany, A., Morita, M., Sano, S., Piotrowski, J., Skrodzka, D., & Slomiany, B. L. (1997). Effect of ethanol on gastric mucus glycoprotein synthesis, translocation, transport, glycosylation, and secretion. *Alcoholism: Clinical and Experimental Research*, ***21*(3),** 417-423.

Suckow, M. A., Weisbroth, S. H., & Franklin, C. L. (Eds.). (2005). *The laboratory rat*. Elsevier, pp100.

Sung JJ ,Kurper E J, El-seraq HB (2009). Systemic review.The global incidence and prevalence of peptic ulcer disease.AlimentpharmcoTher 1:**29 (9)** :936-46.doi.1111/j.1365-2056

Suvik, A. & Effendy, A.W.M. (2012). The use of modified Massion's trichrome staining in collagen evaluation in wound healing study. *Mal J Vet Res* **3**, 39–47

Tamura, M., Matsui, H., Kaneko, T., & Hyodo, I. (2013). Alcohol is an oxidative stressor for gastric epithelial cells: detection of superoxide in living cells. Journal of clinical biochemistry and nutrition, **53(2),** 75-80.

Tseng, C. C., Schmidt, K. L., & Johnson, L. R. (1987). Hormonal effects on development of the secretory apparatus of chief cells. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 253(3), G274-G283.

Uehara, T., Elmore, S. A., & Szabo, K. A. (2018). Esophagus and stomach. In *Boorman's Pathology of the Rat* (pp. 35-50). Academic Press.

Vdoviaková, K., Petrovová, E., Maloveská, M., Krešáková, L., Teleky, J., Elias, M. Z. J., & Petrášová, D. (2016). Surgical anatomy of the gastrointestinal tract and its vasculature in the laboratory rat. *Gastroenterology research and practice*, *2016*.

Velasco-Zamora, J. A., Gómez-Reyes, E., &Uscanga, L. (2016). Are the clinical guideline recommendations on gastroprotection being followed? A review in patients taking nonsteroidal anti-inflammatory drugs. *Revista de Gastroenterología de México (English Edition)*, *81*(3), 121-125.

Vogel HG, Vogel WH (1997). Drug discovery and evaluation (Pharmacological assays).Berlin.Springer Verlag.**2**;486-487

Vos, T., Barber, R. M., Bell, B., Bertozzi-Villa, A., Biryukov, S., Bolliger, I., ...&Duan, L. (2015). Global, regional, and national incidence, prevalence, and years lived with

disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, ***386*****(9995),** 743-800.

Wang, A. Y., &Peura, D. A. (2011).The prevalence and incidence of Helicobacter pylori–associated peptic ulcer disease and upper gastrointestinal bleeding throughout the world.*Gastrointestinal endoscopy clinics of North America*, ***21*****(4),** 613-635.

WadoodA,Ghufran M, Jamal SB, Neem M, Khan K.,et al (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan.Biochem Anal Biochem**2**:144.doi:10.417212161-1009.1000144

Werner, O., & Hack, W. H. (1979). Effects of prolonged administration of metiamide on serum gastrin, gastrin content of the antrum and gastric corpus, and G-cell population in the rat.*Gastroenterology*, ***76***(5 Part 1), 945-949.

Wesonga, L. N. (2017). *Morphological and genotypic characterization of african nightshade cultivars* (doctoral dissertation, university of nairobi).

World Health Organization. (1993). Guidelines for drinking-water quality. World Health Organization.

WHO (2003) b. Traditional, complementary and alternative medicines and therapies .Washington DC.WHO Regional office for the Americas/Pan America Health Organization working group Ops/Oms.

Yao, L., & Bo, H. (2016). Essential Oil Composition of Artemisia scopariaWaldst.&Kitag from Qinghai-Tibetan Plateau of China. *Journal of Analytical Sciences, Methods and Instrumentation*, ***6*****(01),** 1.

Yoshikawa, M., Morikawa, T., Li, N., Nagatomo, A., Li, X., & Matsuda, H. (2005). Bioactive Saponins and Glycosides. XXIII. Triterpene Saponins with Gastroprotective Effect from the Seeds of Camellia sinensis—Theasaponins E3, E4, E5, E6, and E7—. Chemical and Pharmaceutical Bulletin, **53(12)**, 1559-1564.

Zouari, S., Ayadi, I., Fakhfakh, N., Jdir, H., Aloui, L., Kossentini, M., ...&Zouari, N. (2014). Essential oil variation in wild populations of Artemisia saharae(Asteraceae) from Tunisia: chemical composition, antibacterial and antioxidant properties. *Botanical Studies*, ***55*(1),** 1-10.

APPENDICES

Appendix 1: Hematoxylin and Eosin (H & E) Staining Protocol

Description

The oxidation product of haematoxylin is haematin, and is the active ingredient in the staining solution. Haematoxylin is not classified as a dye since the molecule possesses no chromophore. The *in situ* oxidation of haematoxylin is effected by the addition of a strong oxidant to the stain, in this case sodium iodate.

Haematin exhibits indicator-like properties, being blue and less soluble in aqueous alkaline conditions, and red and more soluble in alcoholic acidic conditions. In acidic conditions, haematin binds to lysine residues of nuclear histones by linkage via a metallic ion mordant, in this case aluminium. To ensure saturation of chemical binding sites, the stain is applied longer than necessary, resulting in the overstaining of the tissues with much non-specific background colouration. This undesirable colouration is selectively removed by controlled leaching in an alcoholic acidic solution, (acid alcohol), the process being termed "differentiation". Differentiation is arrested by returning to an alkaline environment, whereupon the haematin takes on a blue hue, the process of "blueing-up". The haematin demonstrates cell nuclei.

Full cellular detail is obtained by counterstaining with the eosin mixture. Colour enhancement is achieved by fortifying the stain with phloxine, a chemical member of the same family as eosin (halogenated fluorosceins). The mechanism of their staining is not fully understood, but is

believed to be of an electrostatic nature. Visualisations most acceptable to the histologist are obtained by applying the dyes in acidic conditions, whereby more intense specific colourations are obtained, the more acidic tissue components taking up the dye to a greater intensity, hence the addition of acetic acid.

Method

1. Deparafinize sections in two changes of xylene for 10 minutes each.
2. Rehydrate sections in two changes of absolute alcohol for 5 minutes each, then 95% alcohol and 75% alcohol for 2 minutes each.
3. Wash the sections in briefly distilled water.
4. Stain nuclei with the alum haematoxylin for four minutes.
5. Rinse in running tap water for five minutes.
6. Differentiate with 0.3% acid alcohol. The end point being the point at which after blueing up, the background is almost colourless.
7. Rinse in running tap water for five minutes.
8. Rinse in Scott's tap water substitute.
9. Rinse in tap water for five minutes.
10. Stain with eosin for 2 mins. Running water removes any over-staining.
11. Dehydrate in two changes of 95% alcohol, then two changes of absolute alcohol for 5 minutes each.

12. Clear in two changes of xylene, 5 minutes each and mount with a xylene based medium.

Results

Collagen	pale pink
Muscle	deep pink
acidophilic cytoplasm	red
basophilic cytoplasm	purple
nuclei	blue
erythrocytes	cherry red

Reagent Formulae

1. Lillie Mayer alum haematoxylin

aluminium ammonium sulphate ---- 200 g

haematoxylin (CI 75290) ---------- 20 g

ethanol ---------- 40 ml

sodium iodate ---------- 4 g

acetic acid ---------- 80 ml

glycerol ---------- 1200 ml

distilled water ---------- 2800 ml

In a 4L Ehrlenmeyer flask, to 1000 mls of the distilled water, add the aluminium ammonium sulphate. Place the flask on a stirrer, turn on the stirrer and allow to mix until the alum dissolves - approximately 15 mins. Remove the flask from the stirrer, allow to cool, add the remaining 1800 mls distilled water – to further cool the solution. Add the haematoxylin powder to the alcohol and dissolve as much of the powder as possible by shaking for a few minutes. Pour the strong alcoholic solution of haematoxylin into the cooled alum solution and stir to ensure all the Hematoxylin powder is dissolved, preferrably overnight. Add the sodium iodate, acetic acid, and finally the glycerol. Mix well, plug loosely and store.

It is appropriate to make up a batch of the required amount, dependant upon the usage rate.

2. Acid alcohol 0.3% Acid Alcohol

commercial grade ethanol ----- 2800 ml

distilled water ---------- 1200 ml

conc hydrochloric acid --------- 12 ml

In a sufficiently large container, add the acid to the water, then add the alcohol and mix thoroughly. The generation of fine bubbles is an indication that mixing is thorough.

3. Scott's tap water substitute

sodium hydrogen carbonate --- 10 gm

magnesium sulphate ---------- 100 gm

distilled water ---------- 5 L

Dissolve the salts in the water. Store stock solutions at room temperature.

4. alc acetified eosin/phloxine TQEH

1% eosin Y (CI 45380) ---------- 400 ml

1% aq phloxine (CI 45405) ----- 40 ml

95% alcohol ---------- 3100 ml

gl acetic acid ---------- 16 ml

Mix the above reagents together, and stir well. The solution keeps well.

Appendix 2: PAS (Periodic Acid Schiff) Staining Protocol

Description: This method is used for detection of glycogen in tissues such as liver, cardiac and skeletal muscle on formalin-fixed, paraffin-embedded tissue sections, and may be used for frozen sections as well. The glycogen, mucin, and fungi will be stained purple and the nuclei will be stained blue.

Fixation: 10% formalin.

Section: paraffin sections at 5 um.

Solutions and Reagents:

<u>0.5% Periodic Acid Solution:</u>

Periodic acid ---------- 0.5 g

Distilled water ---------- 100 ml

Schiff Reagent:

Test for Schiff reagent: Pour 10 ml of 37% formalin into a watch glass. To this add a few drops of the Schiff reagent to be tested. A good Schiff reagent will rapidly turn a red-purple color. A deteriorating schiff reagent will give a delayed reaction and the color produced will be a deep blue-purple.

Mayer's Hematoxylin Solution

Procedure:

1. Deparaffinize and rehydrate sections.
2. Oxidize in 0.5% periodic acid solution for 5 minutes.
3. Rinse in several changes of distilled water.
4. Immerse in Schiff reagent for 15 minutes (Sections become light pink color during this step).
5. Wash in lukewarm running tap water for 5 minutes (Immediately sections turn dark pink color).
6. Counterstain in Mayer's hematoxylin for 1 minute**.**
7. Wash in tap water for 5 minutes.
8. Dehydrate and coverslip using a toluene or xylene mounting medium.

Results:

Glycogen, mucin and some basement membranes	red/purple
Fungi	red/purple
Background	blue

Appendix 3: Masson's Trichrome Staining Protocol

Description: This method is used for the detection of collagen fibers in tissues such as skin, heart, stomach etc. on formalin-fixed, paraffin-embedded sections, and may be used for frozen sections as well. The collagen fibers will be stained blue, the nuclei will be stained black and the background is stained red.

Fixation: 10% formalin or Bouin's solution

Section: paraffin sections at 5 um.

Solutions and Reagents:

Bouin's Solution:

Picric acid (saturated) ---------- 75 ml

Formaldehyde (37-40%) ---------- 25 ml

Glacial acetic acid ---------- 5 ml

Mix well. This solution will improve Masson Trichrome staining quality.

Weigert's Iron Hematoxylin Solution:

Stock Solution A:

Hematoxylin ---------- 1 g

95% Alcohol ---------- 100 ml

Stock Solution B:

29% Ferric chloride in water --------- 4 ml

Distilled water ---------- 95 ml

Hydrochloric acid, concentrated ---- 1ml

Weigert's Iron Hematoxylin Working Solution:

Mix equal parts of stock solution A and B. This working solution is stable for 3 months

Biebrich Scarlet-Acid Fuchsin Solution:

Biebrich scarlet, 1% aqueous --------- 90 ml

Acid fuchsin, 1% aqueous ----------10 ml

Acetic acid, glacial ---------- 1 ml

Phosphomolybdic-Phosphotungstic Acid Solution:

5% Phosphomolybdic acid ---------- 25 ml

5% Phosphotungstic acid ---------- 25 ml

Aniline Blue Solution:

Aniline blue ---------- 2.5 g

Acetic acide, glacial ---------- 2 ml

Distilled water ---------- 100 ml

1% Acetic Acid Solution:

Acetic acid, glacial ---------- 1 ml

Distilled water ---------- 99 ml

Procedure:

1. Deparaffinize and rehydrate through 100% alcohol, 95% alcohol 70% alcohol.
2. Wash in distilled water.
3. For Formalin fixed tissue, re-fix in Bouin's solution for 1 hour at 56^{o}C to improve staining quality.

4. Rinse running tap water for 5-10 minutes to remove the yellow color.

3. Stain in Weigert's iron hematoxylin working solution for 10 minutes.

4. Rinse in running warm tap water for 10 minutes.

5. Wash in distilled water.

6. Stain in Biebrich scarlet-acid fuchsin solution for 10-15 minutes. Solution can be saved for future use.

7. Wash in distilled water.

8. Differentiate in phosphomolybdic-phosphotungstic acid solution for 10-15 minutes or until collagen is not red.

9. Transfer sections directly (without rinse) to aniline blue solution and stain for 5-10 minutes. Rinse briefly in distilled water and differentiate in 1% acetic acid solution for 2-5 minutes.

10. Wash in distilled water.

11. Dehydrate very quickly through 95% ethyl alcohol, absolute ethyl alcohol (these step will wipe off Biebrich scarlet-acid fuchsin staining) and clear in xylene.

12. Mount with resinous mounting medium.

Results:

Collagen	blue
Nuclei	black
Muscle, cytoplasm, keratin	red

UNIVERSITY OF NAIROBI

FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,

Tel: 4449004/44420
Ext.
Direct Line. 4448

Dr Pauline M. Mureithi
C/o Dept of Vet Anatomy & Physiology.

REF:FVM BAUEC/2016/120

Dear Dr Mureithi,

07/11/2016

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

A morphologic and morphometric study on anti-ulcerogenic effects of selected African nightshades (*Solanum nigrum* L.) genotypes in a rat model

By Mureithi Pauline Mbeere J56/75583/2014

We refer to the above revised proposal that you submitted to our committee for review. We have now reviewed the proposal and have noted that you have addressed the issues that were raised in our communication to you dated 07/09/2016 regarding the methods to be used when carrying out the toxicity studies, animal husbandry, restraint and euthanasia of the animals.

We hereby approve your study as detailed in your revised proposal.

Rodi O. Ojoo BVM MSc PhD
Chairman,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine