THE EFFECT OF EXTRACT OF *ALOE VERA* ON ISCHEMIA-REPERFUSION INDUCED MUCOSA INJURY IN THE RAT

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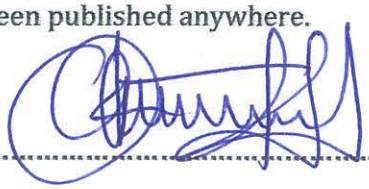
DECLARATION

I KAMBUGU RICHARD a final year student of Kampala International University School of Pharmacy do solemnly submit that this research report is a representation of a lengthy period of dedication, discipline and hard work geared towards the amicable completion of this project. Which is a mark symbolic of the knowledge acquired throughout my four and a half years study for a Bachelors degree of pharmacy and therefore to the best of my knowledge, I hereby declare that this is my own humble effort which has not been published anywhere.

SIGNATURE OF AUTHOR .....  .....**DATE**.....04-03-2014.....

SIGNATURE OF SUPERVISOR.....**DATE**.....

DEDICATION

The legendary late Madiba Nelson Mandela:Truly an icon. A man with the heart of God, one who must have learnt at the feet of Jesus Christ himself. This evident in his selfless nature throughout his entire life and clearly fulfilling the scriptures 'whoever humbles himself will be lifted and who ever lifts himself will be humble(Mattew 23:12). He will always be missed and his monument shall forever tower over others throughout the chronicles of humanity.

The late Esteins: Reading Esteins' profile I have learnt to engage my mind the inside cosmos that God instituted and lies mystical to those who fail to challenge their minds but also a social activist for the lives of the lsealies who were being butchered in Germany and also against the atomic boom proliferation in America later used in Japan. Its only hardwork and service to others that scored giants like him into the rim of achievers not might in the face of conflict or being intellectually elite.

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God, the Joy of my heart , the God of Abraham, Isaac and Jacob, the God of the Bible whose true nature and name equates to Love only Him has blessed me with knowledge and life to see better days and Whose instruction commands my destiny. To Him I ascribe and choose to honor with this research.

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Mom and Dad (I learnt from the best, I learnt from them), they have been the best examples in all ways of life. They have been more than a crutch but a shoulder to which I have leaned all the time and, to my Siblings; they are stuck with me.

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endless expedition that you have began in my life by letting me crawl and find support on to the backs of giants. There is surely no place like home.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES, DIAGRAMS, MAPS AND PHOTOGRAPHS

Tables

Graphs

Photographs

Maps

ACRONYMS

EU	Esophageal Ulcers
GU	Gastric Ulcers
DU	Duodenal Ulcers
PUD	Peptic Ulcers Disease
H_2 RA	Histamine two Receptor Antagonists
AV	Aloe vera
AV-IR	Aloe vera and Ischemic Reperfusion
AVRan-IR	Aloe vera-Ranitidine and Ischemic Reperfusion
Lab	Laboratory
HCl	Hydrochloric Acid
GERD	Gastro Esophageal Reflux Disease
ZES	Zollinger Ellision's Syndrome
BP	British Pharmacopoeia
USP	United States Pharmacopoeia

CHAPTER I

ABSTRACT

Aloe vera is one of the many herbal remedies used in Uganda for the treatment of a wide range of illness ranging from trauma/ wounds to infections even for cosmetic purposes. The research in to its protective role on ischemia induced gastric ulcers was aimed at establishing and confirming its reported efficacy on peptic ulcers, time of onset of action and time taken for an optimum therapeutic response. This research investigated the extent of *aloe vera's* protective properties which were compared with the therapeutic effects of an anti histamine (ranitidine). The phytoextracts (mucilage) of the plant was administered to the test subjects orally by a cunnula immediately prior to ulcer induction, thereafter the subjects were sacrificed and stomach contents examined macroscopically by hand lens. The histopathological data was collected appropriately compiled and computed using a combination of textual, diagrammatic and graphical representations. This data showed that *aloe vera* was effective in the prevention of peptic ulcers. Using a series of statistical tools to ascertain the relevancy of the findings.

1.2.0 INTRODUCTION

Peptic ulcer disease is a disease that manifests with a complex of symptomology resulting from disruption in the integrity of the GI mucosal epithelium causing crate like lesions. (Fauci et al., 2008). This mucosa has three areas of secretion, the cardiac gland area that secrete mucus and pepsinogen, the oxyntic (parietal) gland area, which corresponds to the fundus and body of the stomach, secretes hydrogen ions, pepsinogen, and bicarbonate and the pyloric gland area in the antrum which secretes gastrin and mucus. (Craig et al.,NY)

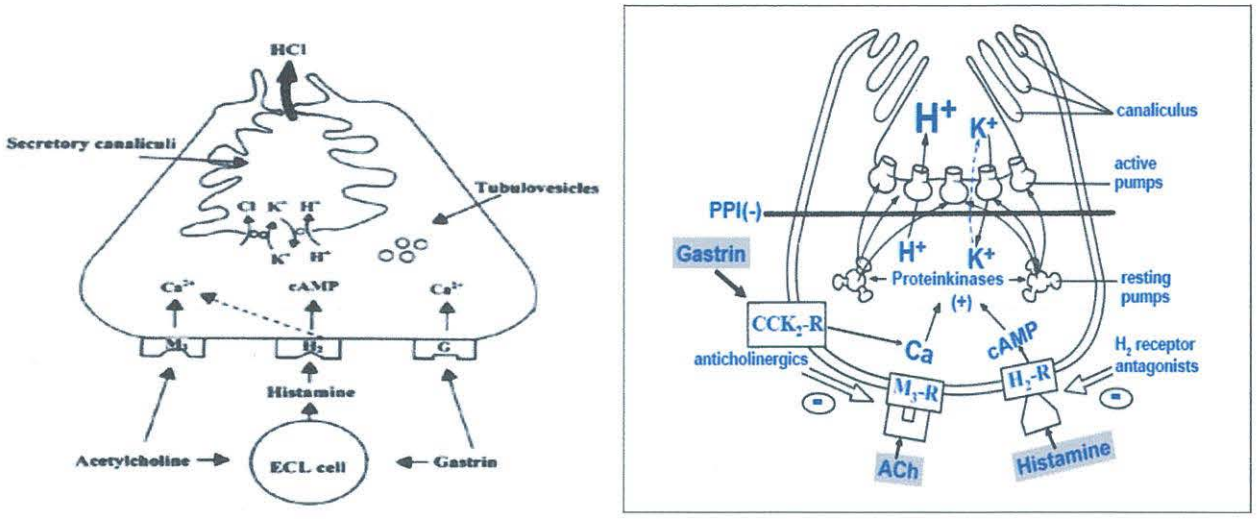

Fig1. Parietal cell (Springer.com)

These lesions are as result of autolysis by pepsin a hydrolytic enzyme and Hydrochloric acid. (Craig et al.,NY) This contributes to the pathogenesis of many other known upper GI diseases for instance, gastroesophageal reflux disease (GERD), upper GI bleeding (Kimble et al., 2009) and Zollinger Ellison's Syndrome (ZES). The disease presents with epigastric pain exacerbated by fasting and improved with meals (Fauci et al., 2008) and is often chronic in nature hallmarked by sensations of burning aching pain, nausea, indigestion, vomiting, loss of appetite or weight loss and hematemesis (Goljan, 2007) normally within the abdominal cavity (the upper quadrants) and is felt before a meal or several hours afterwards. The ulcers are given names denoting the regions affected such as gastric ulcers

(GU), duodenal ulcers (DU), esophageal ulcers (EU) (Kimble et al., 2009) and can be classified as of acute onset, or chronic or severe in some cases involving perforation of the mucosa hence requiring surgery. (Nzalubara, 2005)

The imbalance between the protective factors of the gastric mucosa (bicarbonate, mucin/mucus secretion, mucosal blood flow, apical surface membrane transport, epithelial regenerative capacity and prostaglandin production) and the destructive factors (acid and pepsin) (Kumar et al.,NY) predisposes to peptic ulceration. These factors being secondary to pathological conditions like mucosa ischemia, decreased gastric emptying, pyloric atony for GU and increased vagal stimulation, hormonal drive, parietal cell mass and gastric emptying for DU extra. (Craig et al.,NY). These define the approach to drug therapy which extends from non pharmacological to pharmacological approaches all aiming at the goals of relieving symptoms and signs, healing the ulcers, allowing regeneration of the mucosal layer and treating the bacterial infection known for exacerbating the ulceration (helicobacter pylori). Therefore a range of drugs are used falling in the above categories in the management of peptic ulcers which include those with antihistamine action (Rantidine, pCimetidine), proton pump inhibitors (omeprazole) and protective action (antacids, sucrafate, misoprostal) and others like pirenzepine, antibiotics forinstance metronidazole, clindamycin, ampicillin, tetracycline, amoxicillin extra. (Katzungu, 2009) Herbal extracts/ formulations used include those of *Aloe vera* known to confer anti-inflammatory action while reducing acid secretion and stimulating pepsin secretion. Thus providing pain relief, preventing internal bleeding and aiding ulcer healing. (Whole health md, 2005).

This soothing action of *aloe vera* has long been exploited in European folk medicine to relieve heartburn and ulcers. (Whole health md, 2005).Aloe produces two substances, gel and latex, which are used for medicines. Aloe gel is the clear, jelly-like substance found in the inner part of the aloe plant leaf. Aloe latex comes from just under the plant's skin and is yellow in color. Some aloe products are made from the whole crushed leaf, so they contain both gel and latex. (Kimbali, 2012)The gel is used as topical ointment, creams, lotions and shampoos in which *aloe vera* acts via its antiseptic, anaesthetic, anti-inflammatory,

antipruritic, and moisturizing properties in the treatment of wounds though the contrary is true for deep surgical wounds and radiation burns. (NCCAM Publication, 2012.)

Non pharmacological approaches includes cessation of drinking alcohol, smoking, taking of NSAIDs and prompt taking of meals.

1.2.1 Background

Peptic ulcers result from an imbalance between factors that damage the mucosa and the normal mucosal defense and repair mechanisms. (Kumar et al NY) Examples of factors that predispose to ulceration include *Helicobacter pylori* infection, stomach acid, stress, alcohol, tobacco, and non-steroidal anti-inflammatory drugs (NSAIDs). (Guyton and Hall, 2006) Inhibition of cyclooxygenases (COX) and prostaglandins (PGs) accounts for both the anti-inflammatory effects and the gastrointestinal (GI) toxicities of NSAIDs. Prostaglandins coordinate secretion of protective mucus, surfactant, and bicarbonate, reduce acid secretion, decrease epithelial permeability, increase mucosal blood flow. (Zhu and Kaunitz , 2008; Somasundaram et al 1995)

Damage to the gastric mucosa barrier due to ischemia and reperfusion (I-R) injury is a common and serious condition (Wada et al., 1995). The gastric mucosal injury induced by I-R has been regarded as a useful experimental model for the study of stress ulcer formation (Kitano et al., 2005).I-R usually results from conditions such as circulatory shock, trauma, sepsis, circulatory insufficiency or thrombosis (Wada et al., 1995; Kubes et al., 1992).

Considerable evidence has accumulated to show that, generation of reactive oxygen species, such as superoxide, hydrogen peroxide and hydroxyl radicals, by invading neutrophils mediate the injury associated with I-R (Andrews et al., 1992; Panes et al., 1999). Oxygen derived free radicals generated during reperfusion initiate a series of events that causes mucosal damage and disruption of the barrier. As a result, a number of pharmacological interventions are under development to prevent the cascade of events that eventually leads to the gastric mucosa barrier being compromised. These include

application of anti-oxidants and use of drugs to block the effect of inflammatory mediators and acid (Kitano et al., 2005; Kitano et al., 1997).

Histopathological aspect

Gastric and duodenal ulcers have a similar gross and microscopic appearance. These lesions penetrate the mucosa into the submucosa, and often into the muscularis propria or deeper. They appear as rounded and sharply punched-out craters 2 to 4 cm in diameter. (Kumar et al NY)

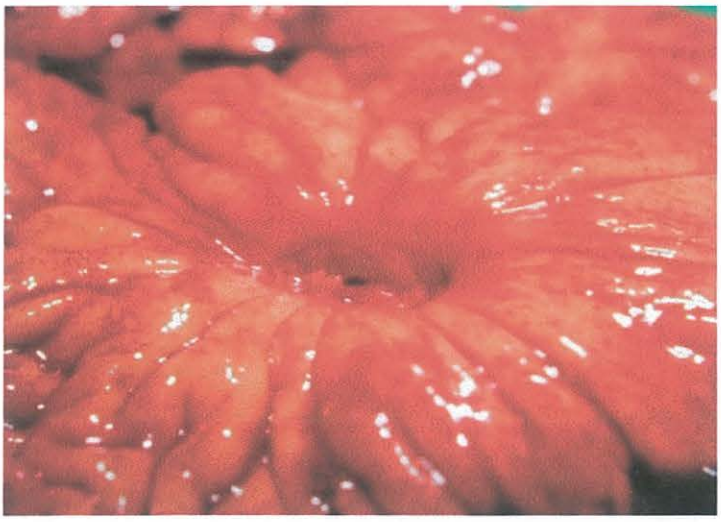

Fig 2. Peptic ulcers (leahculynn, 2014)

They also vary in size with locality: duodenal ones tend to be smaller than gastric lesions found mostly on the anterior and posterior walls of the first portion of the duodenum and the lesser curvature of the stomach. Occasional gastric ulcers occur on the greater curvature or anterior or posterior walls of the stomach. (Kumar et al., NY)

The margins of the ulcer crater are perpendicular with some mild edema of the immediately adjacent mucosa and no significant elevation or beading of the edges seen with gastric cancers. (Kumar et al., NY)

The surrounding mucosal folds may radiate like wheel spokes. The base of the crater appears remarkably clean, as a result of peptic digestion of the inflammatory exudates and

The drug may also be associated with significant nausea in palliative care patients. (Therapeutic Guidelines Complete, 2007)

Aloe vera

Fig.4 Aloe vera plant (Aloe vera.com, 2013)

It is a succulent plant (Liliaciae Sub species aloinae), a member of the lily and onion family (Atherton, 1997), whose genus includes herbs, shrubs and trees, bearing spikes of white, yellow or red flowers. Aloe leaves are fleshy, are strongly cuticularized and are usually prickly at the margin. (Saunders et al., 2002) Aloe is also related to garlic and asparagus, of which there are more than three hundred varieties but only a few have medicinal properties. *Aloe Vera* Barbadensis Miller is the most potent. The name *Aloe Vera* or True Aloe probably stems from the Arabic word Alloeh meaning "Shining bitter substance". For thousands of years *Aloe vera* has been used and referenced in various literatures by various cultures (Egyptians, Greeks, Romans, Indians, the Chinese, Assyrians, and Mediterranean civilizations, as well as in Biblical times). (Atherton, 1997). For instance dating 6,000years back *Aloe vera* was engraved on stone carvings in Egypt as the plant for immortality presented as burial gift to deceased pharaohs. (Grundmann, 2012) Its medicinal purpose

necrotic tissue. Infrequently, an eroded artery is visible in the ulcer (usually associated with a history of significant bleeding). (Kumar et al., NY)

The histologic appearance varies with the activity, chronicity, and degree of healing. In a chronic, open ulcer, four zones can be distinguished (1) the base and margins have a thin layer of necrotic fibrinoid debris underlain by (2) a zone of active nonspecific inflammatory infiltration with neutrophils predominating, underlain by (3) granulation tissue, deep to which is (4) fibrous, collagenous scar that fans out widely from the margins of the ulcer. Vessels trapped within the scarred area are characteristically thickened and occasionally thrombosed. (Kumar et al., NY)

However acute stress ulcers are circular and small (<1 cm in diameter). The ulcer base is frequently stained dark brown by the acid digestion of extruded blood. Unlike chronic peptic ulcers, acute stress ulcers are found anywhere in the stomach occuring singly, but more often there are several, located throughout the stomach and duodenum. (Kumar et al., NY)

Rantidine

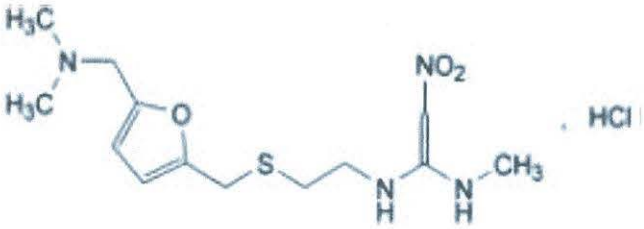

Fig 3. Chemical structure of ranitidine hydrochloride. (BP, 2009)

Ranitidine is a H2 histamine receptor antagonist available as ranitidine Injection, Oral solution and tablets (150mg, 300mg), syrups 2-3mg/kg . (BP, 2009; USP, 2007)

Has anticholinergic activity; the anticholinergic effects of drugs are additive and all contribute to the 'anticholinergic load' producing delirium in patients on antipsychotics.

was orally as a laxative and topically for skin conditions and wound healing. The plant is native to North Africa but a now is extensive cultivated worldwide because of its adaptability to varying climatic conditions and easy cultivation. (Grundmann, 2012)

A variety of aloe species are still used in folk medicines of Africa and Asia. Hunters in the Congo reportedly rub their bodies in the clear mucilaginous gel to reduce perspiration; some African tribes apply the gel for chronic conjunctivitis; the gel is used in India for the treatment of asthma. (Grundmann, 2012)

Aloe vera products are derived from the mucilage located in the parenchymatos cells of the *aloe vera* leaf while aloes is a solid residue originating from the aloetic juice of the pericyclic obtained by evaporating the liquid which drains from the transversely cut leases. (Saunders et al., 2002) Contemporary use of these products as folk/ traditional remedy is in the treatment of diabetes, asthma, epilepsy, osteoarthritis. It's included in topical formulations for instance lotions and creams for the treatment of osteoarthritis, burns, sunburns, psoriasis and as a pain killer. (Grundmann, 2012)

The key to its therapeutic efficacy lies in the wide range of its primary and secondary metabolites contained in the mucilaginous gel and latex, the latter is exploited for it strong laxative and purgative effects. In attempts to understand the way the plant brings about these effects a series of mechanisms of action have been postulated as listed below: (Grundmann, 2012)

Proposed Mechanisms of Action (Grundmann, 2012)

- Stimulation of macrophage and fibroblast activity, increased collagen and proteoglycan synthesis
- Mannose-6-phosphate binds to growth factor receptor on fibroblasts and enhances their activity
- Macrophage activation through increased nitric oxide synthase activity by acemannan, leading to release of fibrogenic cytokines

- Upregulation of phagocytosis and fungicidal activity of macrophages by acemannan
- Acemannan and other cell wall biomaterial may promote stability of growth factors and prolong
- Stimulation of granulation tissue
- Inhibition of Thromboxan A_2
- May promote hypoglycemic effect by normalizing membrane-bound enzyme activities of phosphatases and hydrolases and increased glucose metabolism; potential active compounds include the phytosterols lophenol, cycloartenol and their alkylated derivatives
- Anti-inflammatory effect of plant sterols like lupeol, campesterol, and β-sitosterol through bradikinase activation, prostaglandin F2 and E2, as well as thromboxane A2 inhibition and inhibition of IL-10 secretion
- Inhibitory effect on release of reactive oxygen species from human neutrophils by reducing intracellular free calcium levels
- Increase in mRNA expression of metalloproteinases and plasminogen activator may lead to angiogenic activity in endothelial cell

1.2.2 Problem statement

The incidence of peptic ulcer is 0.10% to 0.19% (Sung et al., 2009) and continues to rise with about 20-40% (Sung et al., 2009) people having to suffer from peptic ulcers in their life. The available medicines relieve / cure but with relapses occasionally in almost every sufferer not forgetting to mention the side effects and interactions with other medicines that have proven fatal in certain instances. There is however claimed therapeutic efficacy of aloe vera to topical and enteric ulcers, thus a number of modes of action have been stated in trying to understand the way the plant works to modify body physiology but still more work is required on the subject particularly gastric mucosal injury induced by Ischemia as regarded useful by Kitano et al,(2005).This research was actually to test for the therapeutic efficacy of *aloe vera* in terms of protection against ischemia stress induced ulcers.

1.2.3 General Objective

The research was to investigate the claimed efficacy of *aloe vera* against peptic ulceration, ascertain the extent of the protective role played by *aloe vera*, compare the protective functions of *aloe vera* with modern conventional medicines for instance ranitidine, illuminate on the mode of action of *aloe vera* as a gastrointestinal protecting drug, define the required dose to arrive at the anticipated function and to point out / state any evidence for the toxic profiles of the drug in doing the protective function.

1.2.4 Specific objectives

- To investigate the protective role of *aloe vera* and compare this role to the therapeutic outcome of ranitidine as a drug used in the management of peptic ulcers.
- To investigate the function of histamine in mucosa protection against its role in acid secretion and compare this role to the role played by *aloe vera*.
- To investigate the synergic function that may exist when ranitidine is given concurrently with *aloe vera* gel prior to ulcer induction in the test subjects.

1.2.5 Research questions

These were;

- Does *aloe vera* protect against ulceration?
- What is the mechanism of protection?
- Does the moisturizing / hydration effect if the gel play an effect of altering mucosal ischemia?
- Does *aloe vera* potentiate mucus protection due to its gel nature?

1.2.6 Hypothesis

Aloe vera has a protective function against stress induced ulcer by ischemia which reduces susceptibility to any acute stress and interferes with the active transport and secretion of Hydrogen ions into the mucosa.

1.2.7 Scope of the study

- Extent / magnitude of mucosal damage
- Effect on gastric acid secretion and mucosal integrity

1.2.8 Significance /justification

- The research was to ascertain the preventive action of aloe *vera* on ischemia reperfusion peptic ulcers.
- The research was to provide basis for use of *aloe vera as* alternative drug because of side effects of other anti ulcer medication.
- The research was going to ascertain the toxic properties during its action in preventing peptic ulceration.

CHAPTER II

2.1.0 LITERATURE REVIEW

The prevalence of peptic ulcers is One in 10 Americans, these affecting people of over 30years. Children are less affected compared to adults well as duodenal ulcers affect men are more than women. People in the age groups of 60 and 70 are much more affected by gastric ulcers. (Whole health md, 2005). This translates to about 4million individuals being affected per year in the United States with a life prevalence of proximately 12% in men and 10% in women. Ultimately peptic ulcers accounts for a mortality rate of 15000deaths per year causing an estimated financial burden of proximately $10 Billion in the United States. (Fauci et al., 2008) Studies from Europe and US revealed an annual incidence of PUD ranges from 0.10% to 0.19% based on physician diagnosed PUD and from 0.03 to 0.17 % based on hospitalization data. (Sung et al., 2009)

A number of researches have been done in this area testing the efficacy of *aloe vera* as proven alternative medicine in the treatment of peptic ulcers and also the importance of other factors in the pathogenesis of the disease which appear to be influenced or enhanced by the chemical components in *aloe vera* phytoextracts to a curative and protective outcome for instance:

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used for the treatment of pain, fever, and inflammation. Long-term use of these drugs is associated with significant gastric injury. Activated neutrophils and oxidative stress seem to play a significant role in NSAID-induced gastric mucosal damage. (Wagner et al., 2012) A research study was carried out to ascertain the protective effects of an antioxidant and anti inflammatory enzyme heme oxygenase-1 (HO-1), in NSAID-induced gastric injury. Indomethacin injected intraperiteonially caused gastric inflammation and ulcers, neutrophil activation, and increased tissue expression of interleukin-6 and tumor necrosis factor-alpha in mice. Inducing HO-1 with cobalt protoporphyrin reduced gastric inflammation, number of stomach ulcers, tissue neutrophil activation, and proinflammatory cytokine expression caused by indomethacin. These findings suggested that the induction of an anti-inflammatory and cytoprotective enzyme HO-1 may be a strategy to overcome the gastrointestinal adverse effects limiting the use of NSAIDs. (Wagner et al., 2012)

Another research was carried out to investigate the protective role of mast cells and therefore histamine against the development of peptic ulcers. Mast cell deficient mice were used. They reported that mast cell-deficient mice had extremely high incidence of severe peptic ulceration when exposed to the NSAID piroxicam. (Fritz, 2011) This enhanced ulcer susceptibility could be reversed by reconstitution with mast cells. Furthermore, wild type

mice treated with diphenhydramine hydrochloride, a commonly used antihistamine that blocks histamine H1 receptors, developed a similarly high incidence of peptic ulcers following piroxicam exposure. The protective effect of mast cells is independent of TNF, blockade of H2 receptors, or acid secretion. These data indicated a critical role for mast cells and the histamine that they produce in prevention and repair of piroxicam-induced gastric mucosal injury. (Fritz, 2011)

Mast cells and leukocytes do play a role in the pathogenesis of peptic ulcers via the stimulation of angiogenesis and secretion of proinflammatory mediators TNF –alpha, IL-10 and histamine. A research to compare the effects of *Aloe vera* and sucralfate on gastric microcirculatory changes, cytokine levels and gastric ulcer healing was carried out using 48 male spraque-dawley rats divided into four groups. Administration of 20% acetic acid induced gastric inflammation, increased leukocyte adherence in postcapillary venule and TNF-alpha level and reduced IL-10 level. Aloe vera treatment reduced leukocyte adherence and TNF-alpha level, elevated IL-10 level and promoted gastric ulcer healing (Eamlamnam. et al., 2006).

A research carried out on 17 patients in the 1960s found out that treating these subjects with Aloe vera provide relief from peptic ulcers though there was no comparison with a placebo. The same study was carried in1993 on stress acclimatized rats; aloe extracts suppressed the ulcerogenic effects of stress. Further studies indicated that certain compounds in aloe vera reduced the secretion of stomach juices in mice and rats and protected them against the formation of lesions. (Whole health md, 2005).

Another research was carried out using aloe vera gel as sole medication with complementary administration of Pro-Banthine in instances of overwhelming distress being indicated to restrain hydrochloric acid secretion. These patients were of age group 24-84 years. The preliminary findings indicated evidence of healing and in one year the lesions were completely healed as confirmed by roentgenographic examination. (Blitz et al., 1963)

A research using varying doses of ethanol extract of *Aloe vera* (Liliaceae) to prevent acutely induced gastric mucosal lesions induced with 0.6M HCl . Gastric acid output was studied in the pylorus ligated and lumen perfuse rats, respectively by titration of the collected gastric juice to pH 7.0. Intraperitoneal injection of *Aloe vera*, dose dependently inhibited gastric acid secretion more effectively at a lower concentration which ultimately meant that *Aloe vera* was endowed with gastric acid anti-secretory activity and could protect the gastric mucosa at low concentrations against injurious agents. (Yusuf et al., 2004)

The study to evaluate the gastro protective effect of Livina, a polyherbal formulation on ethanol (50%) induced gastric ulcers in male Swiss albino mice was carried out.. The results indicated that polyherbal formulation significantly decreased the ulcer index and secretion of stomach acid. (Darbar et al., 2013)

CHAPTER III

3.1.0 METHODOLOGY

The experimental tests were performed on live subjects using Wistar laboratory animals of both sexes acquired by purchase from those reared in the Pharmacy laboratory. Thirty three animals were used whose weight was got by weighing each of the animals ensuring that all test subjects lye within the weight range of 150-200g. The venue for the study was the pharmacology laboratory of Kampala International University. The experiment commenced by fast equipment and material collection and organization. This involved the preparation of 0.1M NaOH solution by weighing 0.1g of the pellets and preparation of phenopthalein indicator solution, purchase and dilution of drugs that is to say ranitidine and ketamine to be used as positive control and anaethestic respectively. These were bought from MM pharmacy at Ishaka trading center. Lastly was extraction and dilution of AV mucilaginous mixture of the gel and latex prior to the commencement of the experiment. Then dose optimization of the above drugs and final administration of the drugs to the test subjects and performing procedures tracheotomy and laparatomy of the subjects and ligation of celiac artery cutting the stomachs out and exposing their contents.

3.1.1 Materials and chemicals

Plant Material

The plant extract was prepared by chopping *Aloe vera* (Liliaceae) leaves into tiny pieces that were later crashed into a paste using a blender from which was squeezed the phytoextract. The extract was then filtered using membrane filters and the resultant solution mixed with 98% ethanol. The concentrate was prepared by evaporating the solution using a water bath maintained at a constant temperature of 40°C (Brian and Turner, 1975). 50mg/kg of residue/concentrate was diluted with 25mls of demineralised water to doses 25, 50, 100 and 200mg/kg calculated according to body weight of the individual animals. Volume of administration was 1 ml per 100 g of body weight. (Yusuf et al., 2004)

Chemicals

Ketamine, sodium hydroxide (NaOH), phenopthalein indicator, Syringe needle gauge 16 or the biggest gauge, Aloe vera extract, Dissecting set with good scissors and surgical knives.

The dose of ranitidine was prepared from ampoules containing 50 mg/2 ml.

3.1.3 Experimental Design

The animals were randomly divided into eight groups and each group consisted of five rats.

Group 1(NS): It represented the sham control group. Normal saline was administered orally and the animals were not subjected to I-R.

Group 2 (NS-IR): Animals in this group were subjected to I-R and administered with normal saline orally.

Group 3 (Ran-IR): The animals were pretreated with ranitidine (50mg/2mls, intraperitoneally) with I-R.

Group 4 (AV-IR): *Aloe vera* extracts (A-25, B-50, C-100 and D-200mg/kg) was administered orally 30 minutes before the animal were subjected to I-R.

Group 5 (AVRan-IR): The animals were administered with (E-25, F-50, G-100 and H-200mg/kg) *Aloe vera* extract 30 minutes before administration of ranitidine (25mg/kg, intraperitoneally) and subjected to I-R.

3.1.3 Experimental procedure

The research was conducted using Wistar rats of weight which were divided into five major group and 8 subsidaries for Group 4 and 5, each contained 3 animals. These male and female Wistar rats were purchased from the animal house of the pharmacy laboratory and pharmacology laboratory. They weighed 150-200g at the time of the experiment. They were housed in groups of five in large cages with mesh bottoms to prevent coprophagy and acclimatized to standard laboratory conditions (25 ± 2°C, 12 hr light/dark cycle).The animals were fed on standard laboratory chow and allowed free access to tap water *ad libitum*. They were randomly distributed into different experimental groups of five rats per group each consisting of 3animals. All animals were deprived of food but not water 24 hours before the experiments.

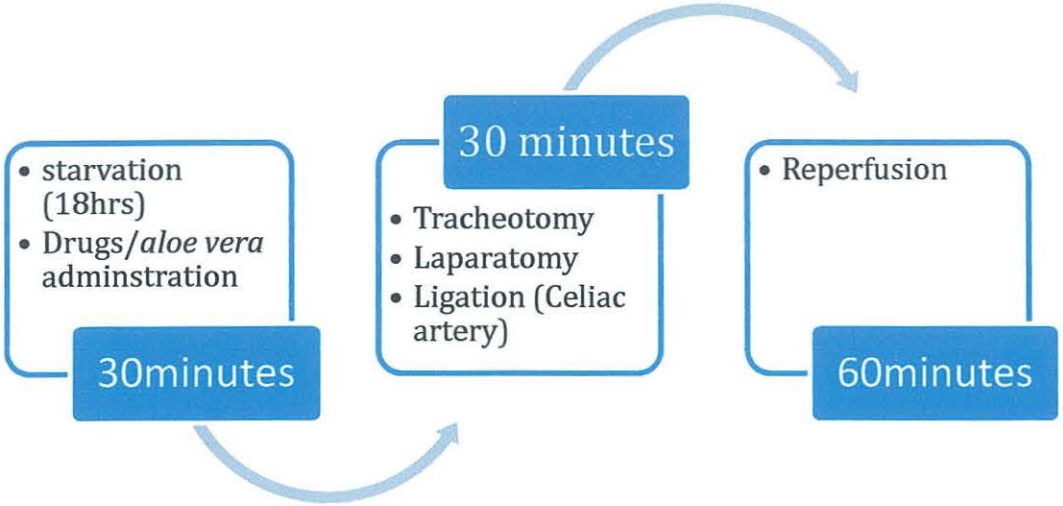

Fig 4. Schematic experimental process.

Aloe vera (3 doses given i.p or intragastric) was given 30mins before ulcer induction and in some cases, ranitidine was administered to serve as positive control. Ulcers were induced experimentally by ligation of the celiac artery for 30mins and then allowing reperfusion for 60mins. At the end of the experiments the animals' stomach were ligated at the pylorus and 2mls of normal saline injected close to the cardiac sphincter then the animal were sacrificed and their stomachs removed, washed and opened along the lesser curvature. Contents were collected on a petric dish and the level of damage was quantified by the determination of size and extent of ulceration, and acid secretion studies. (Yusuf et al., 2004)

3.1.4 Ischemia-Reperfusion of Gastric Mucosa

Gastric mucosal injury was induced by the ischemia-reperfusion (I-R) technique of Wada et al (Wada et al., 1995). Briefly, the rats were anesthetized with ketamine (50mg/kg i.p.) and tracheotomized.

A midline laparatomy was performed, the stomach exteriorized and the pylorus ligated. Ischemia was induced by clamping the celiac artery using a surgical suture for 30 minutes. Reperfusion was established by removal of the clamp. After a 60 minutes reperfusion period, the animals were sacrificed by cervical dislocation. The stomach was removed and opened along the greater curvature. The gastric juice was collected in 5ml Ependoff tubes.

3.1.5 Gastric Mucosal Damage

The severity of gastric mucosal damage was graded according to the length of the lesion as follows; Grade 0 = no visible lesion; grade 1 = hemorrhagic lesions of <1mm; grade 2 = hemorrhagic lesions of 2-4mm and grade 3 = hemorrhagic lesions of >4mm. The ulcer

index for each animal was calculated as the total number of lesions multiplied by their grade (stroff et al., 1996). In sham-operated animals (control), the abdomen was opened and the celiac artery manipulated without clamping.

3.1.6 Measurement of Gastric Secretion

Gastric function was determined by measuring the level of gastric acid of in all the groups at the end of the experiments. Gastric acid level in the gastric juice was determined by titration with NaOH (0.1g/L) and the results expressed as mEq/L.

The ulcer index for each group was calculated by multiplying the number of rats in each grade by the grade number (Yusuf et al., 2004). The curative ratio was then calculated using (Darbar et al., 2013)

Curative ratio = (Control ulcer index) – (Test ulcer index) X 100 / (Control ulcer index)

CHAPTER IV

4.1.0 RESULTS

Secretory studies

Gastric juice and therefore basal acid secretion was significantly different between groups but did not vary much between animals receiving the respective 25, 50, 100, 200mg doses of aloe vera within the test groups (G4 and G5). That is to say G1(NS), G2(NS-IR), G3(Ran-IR) being the control groups and G4-AV-IR { A(25mg/kg), B(50mg/kg), C (100mg/kg), D(200mg/kg)} and G5-AVRan-IR { E,F,G and H had the same dose of aloe vera extract as corresponding groups in G4 in alphabetical order and same single dose of Ranitidine 25mg/ml as in the G3 ranitidine control group.

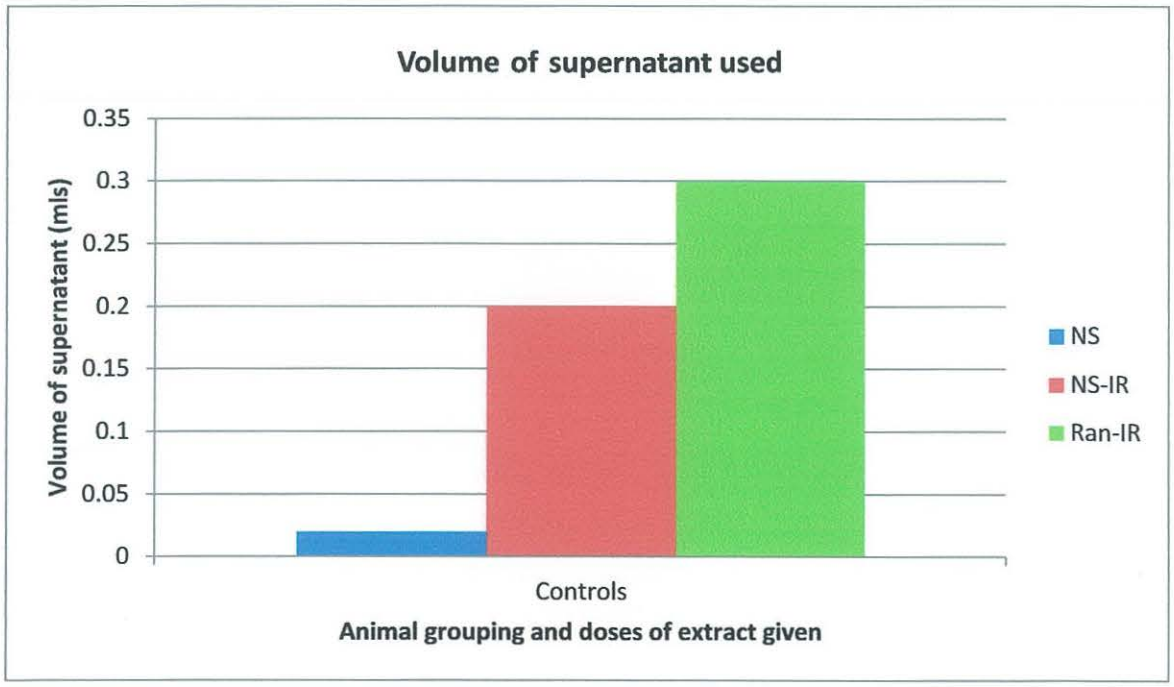

Fig 1. Bar graph that compared control volumes of supernatant that reacted to neutralize NaOH

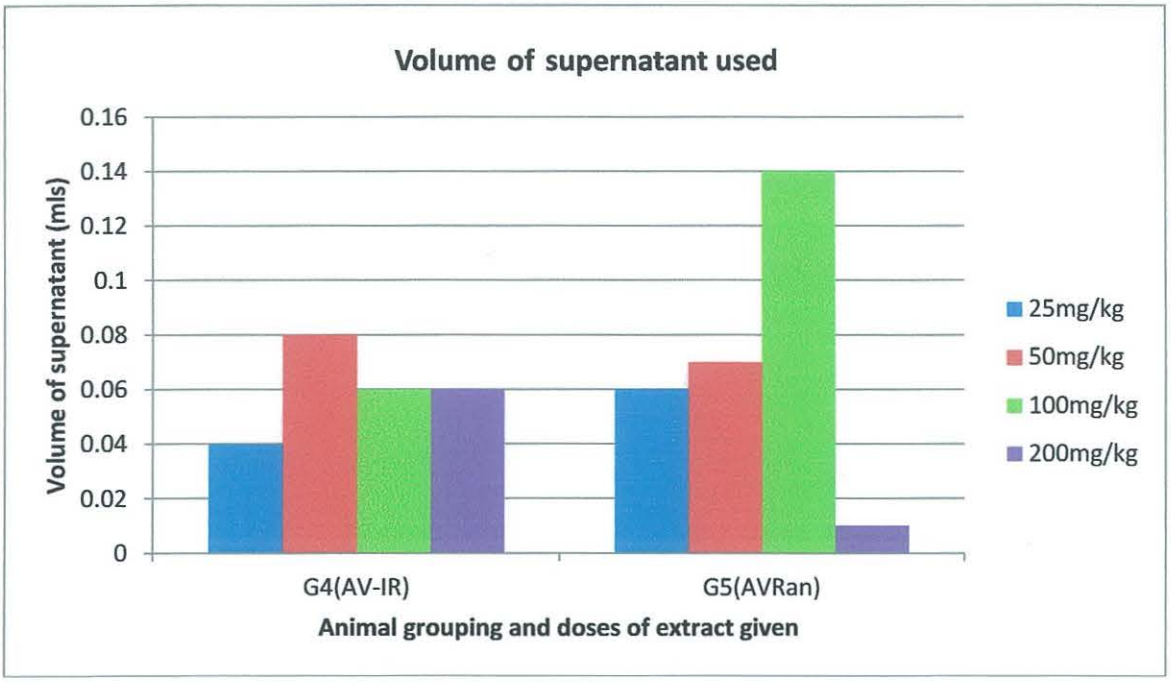

Fig 2. Bar graph that compared test volumes of supernatant that reacted to neutralize NaOH

After 60mins of reperfusion gastric acid was highest in the 200mg (H) group and lowest in the Ran-IR group. A, C, D, E, and F subsidiary groups (within G4 and G5) had intermediate level of acid secreted. Acid secretory inhibition by the extract was dose dependent with significant inhibition even at lowest concentration of extract increasing steadily and exponentially as dose was increased followed by a steady fall with further dosage increment and leveling off at 200mg concentration and falling exponentially in groups G4 and G5 respectively as seen in fig 3 and 4. This was comparatively manifested with the reference convention drug (Ranitidine) but with steady sharp inhibitory rates/ fall in acid levels in the supernatant (fig. 1).

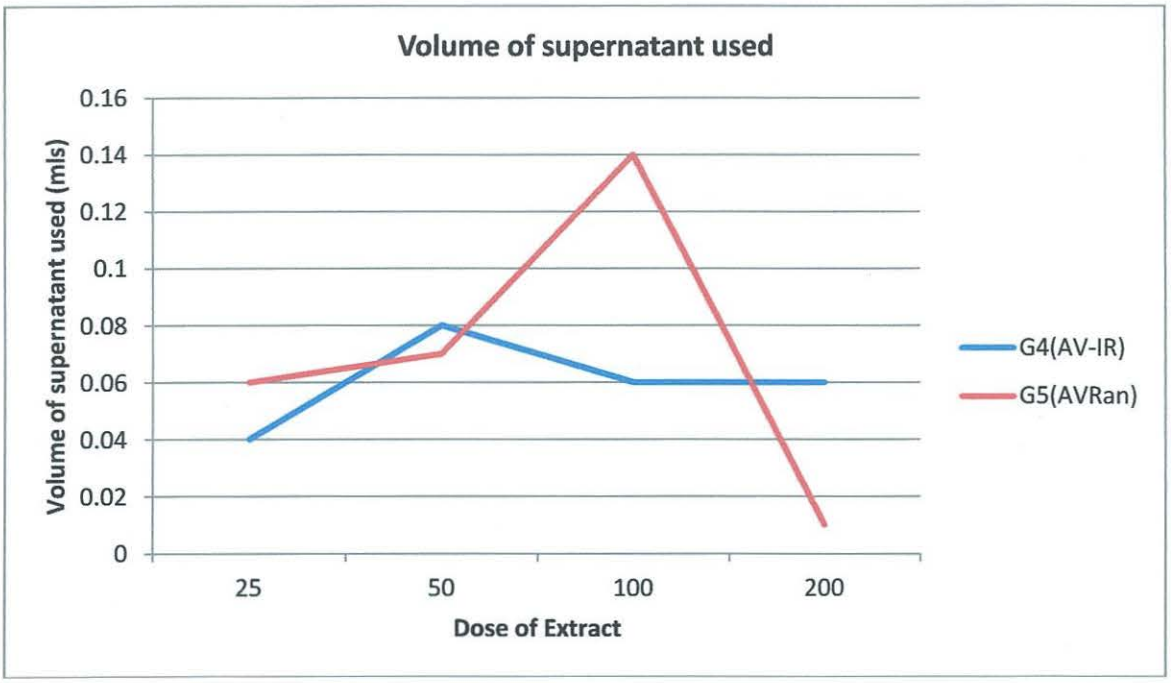

Fig 3. Line graph that compared test volumes of supernatant that reacted to neutralize NaOH

When the two (AV and Ranitidine) were used a small but profoundly stronger eventual reduction in acid secretory volumes was seen using the same corresponding volumes and strengths of AV together with the same Ranitidine single dose used in Ran-IR group though later there was a sharper rise in acid concentration because of a reduction in activity of the two drugs.

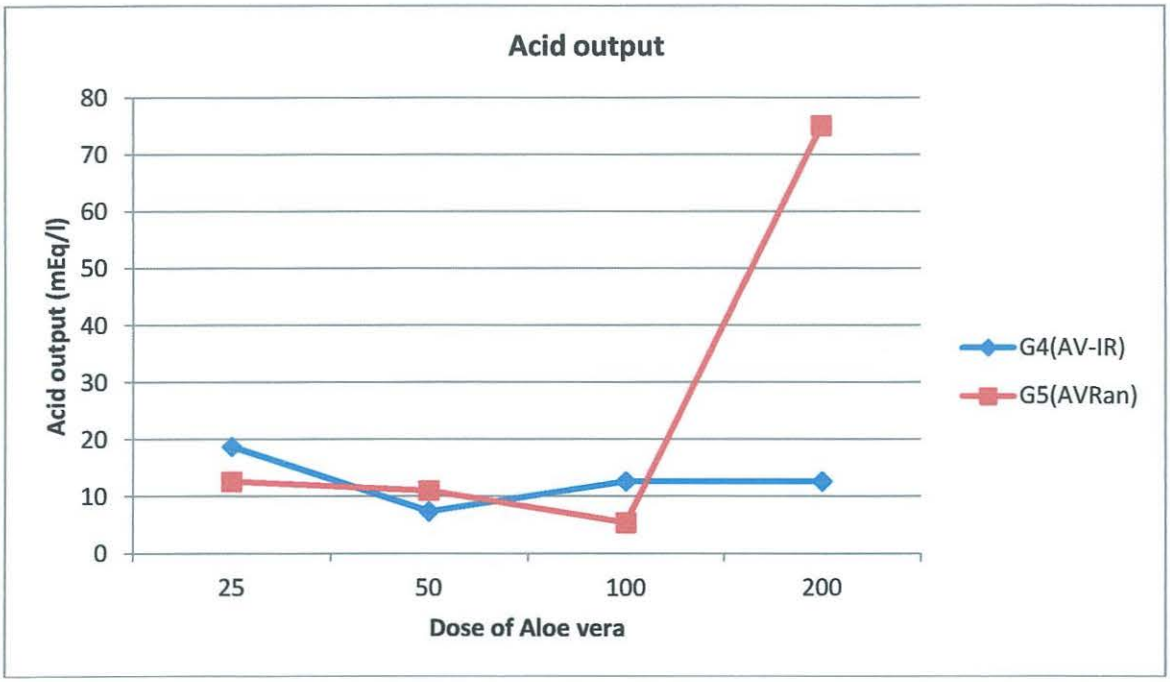

Fig 4. Line graph that compared acid output (mEq/L) present in supernatant that reacted to neutralize NaOH

There was a 10 fold increment in gastric acid levels among the members of NS group against those of NS-IR and a 15 fold increment against members of Ran-IR group while the comparison between groups Ran-IR and G4(AV-IR) showed a slight difference in the inhibitory action that is to say by 3times. On the other hand the difference between G4(AV-IR) and G5(AVRan-IR) was small with the G5 activity starting off at a higher rate of action than that of G4 but with a lower initial maximum effect as compared to G4. Later the G4 group shows a constant trend in terms acid inhibitory effect which ultimately fell completely.

Statistically all doses of the extract registered a fundamental difference in basal acid output

Gastroprotective activity

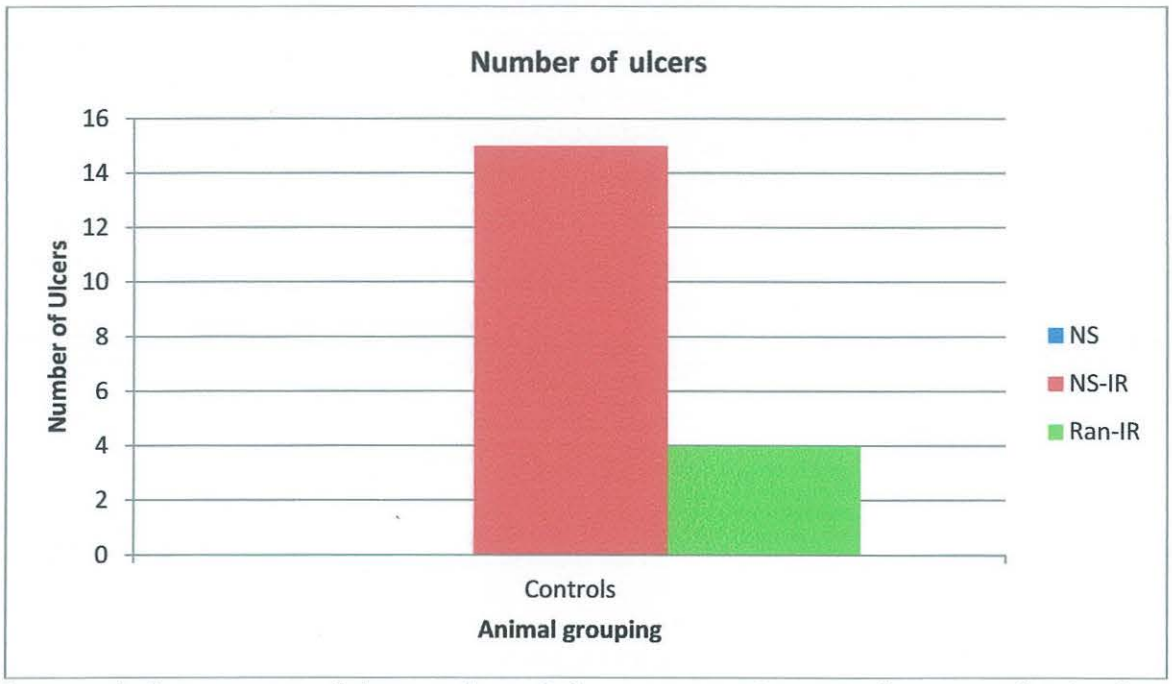

Fig 5. Bar graph that compared the number of ulcers present in control groups of animals

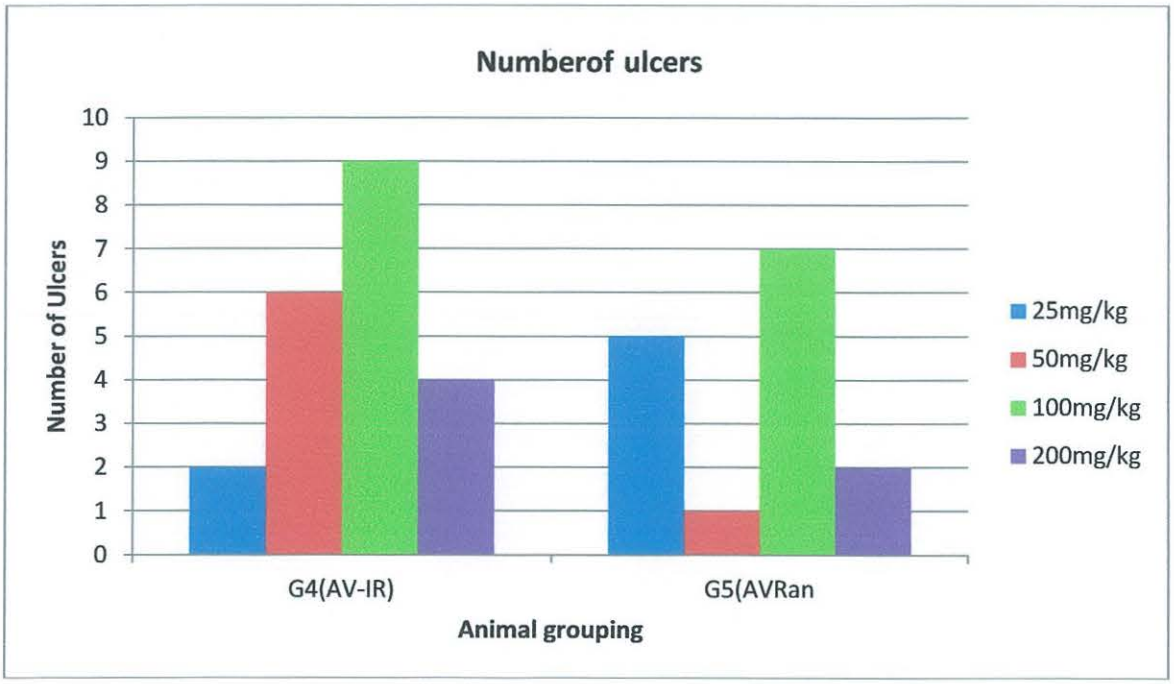

Fig 6. Bar graph that compared the number of ulcers present in test groups of animals

Normal Saline (NS) group

These showed 2-3 reddish patches on a clearly pinkish mucosa.

Normal Saline-Ischemia Reperfusion (NS-IR) group

Macroscopically visible numerous ulcers seen as red colored blood spots on the mucosa with varying sizes and widely distributed all over the mucosal surface was evident.
They varied in length by approximately 0.1-0.4 cm. These ulcers were longer than wide appearing as straight tiny red longitudinal stripes, clean evenly distributed without cellular debris and signs of necrosis but with un clearly bounded areas of surrounding un ulcerated mucosa.
There was evidence of inflammation seen by widely spread protrusion of blood vessels and reddening of the mucosal areas adjacent to the ulcer evidenced by the unusual stretching out of the mucosal folding.

Ranitidine-Ischemia Reperfusion (Ran-IR) group

These animals had a pinkish mucosa with 4 clearly distinct ulcers as long as wide (almost rounded in shape 0.1cm in diameter) and with no protruding blood vessels.

Group 4(Aloe vera and ischemia reperfusion group -AV-IR)

The animals in this group were divided into four groups: group A received 25mg, group B received 50mg while groups C and D received 100 and 200mg/kg doses of aloe vera respectively.

Group A had 2 clearly pronounced ulcers measuring 0.4 x 0.2cm and 0.2 x 0.1cm and several other tiny ulcers covering the entire mucosa in dense mucus secretions. The whole mucosa appeared ulcerated evidenced by the reddish coloration covering the mucosa.

There was wide spread inflammation with less folding of the mucosa as compared to control NS group.

Group B had 6 prominent ulcers measuring 0.2cm in length, sharply demarcated with evidence of bleeding. There was slight inflammation manifested by the prominent blood vessels and less intense general reddening of the surrounding areas.

Group C had numerous ulcers about 9 ulcers on average on the mucosal surface ranging from 0.2-0.3cm in length. These were non perforated, clean, slightly reddened pronounced ulcers with clearly punctuated non ulcerated mucosal adjacent areas. They appeared as straight rather than wide slightly inflamed areas on pinkish mucosa.

Group D had 4 ulcer with length between 0.3 and 0.4 cm. Morphologically the ulcers were less prominent of all groups and deeply reddened in color. Generally the mucosa was pinkish grey in color with less mucus membranes.

Group 5(Aloe vera Rantidine and Ischemia reperfusion group--AVRan-IR)

Test subjects here were divided into groups E, F, G and H whose members received the same doses of aloe vera orally as in G4 (25, 50, 100, 200mg) above plus a dose of ranitidine given intraperitoneally as in the Ran-IR group respectively.

Group E had pinkish red mucosal lining with 5 ulcers of varying length measuring between 0.2-0.3cm.

Group F had one ulcer on a pinkish mucosa

Group G showed 7 ulcers of varying length some between 0.1-0.2cm and some between 0.3-0.4cm on to a predominately redden mucosal lining with protruding blood vessels.

Group H consisted of 2 prominent ulcer seemingly rounded with diameter 0.3cm and bleeding, the mucosa generally appeared pinkish red.

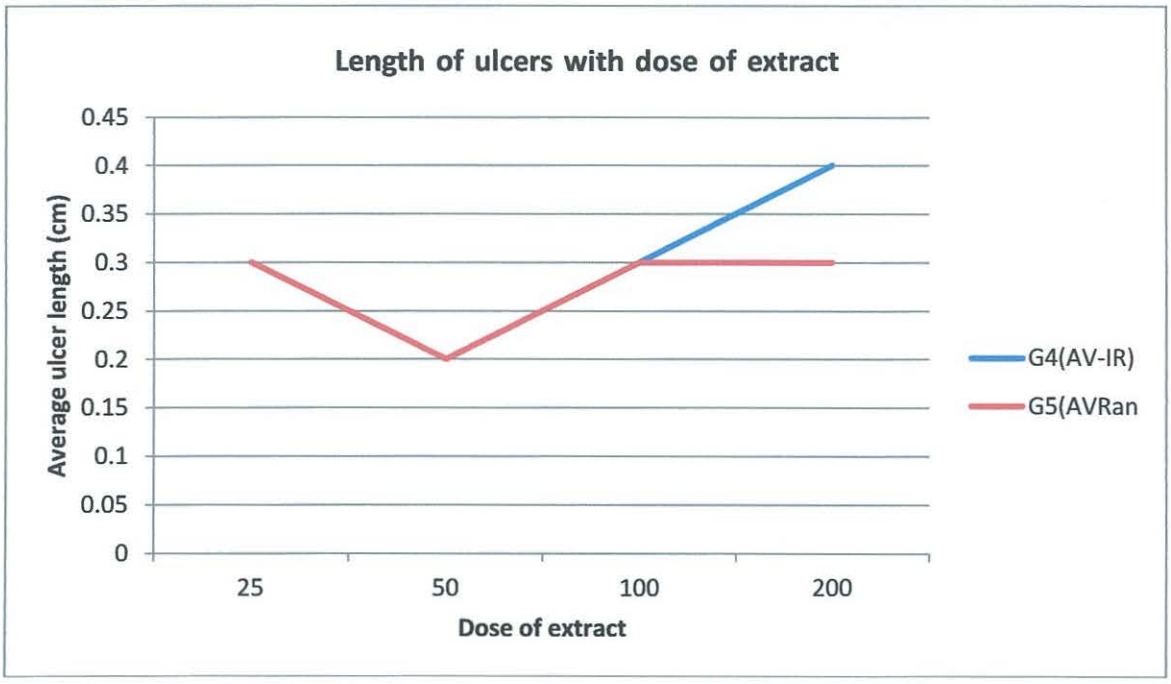

Fig 7. Line graph that compared the number of ulcers present in test groups of animals.

The ulcer index for each group was calculated using the formula:

total ulcer number of each rat in each grade x the grade number (Stroff et al., 1996) average ulcer indices was (4,12, 18, 16) for Group 4 (G4) and (10, 2, 21,4) for Group 5 (G5) in the respective dosage groups of extract 25, 50, 100 and 200mg.

The curative ratio was then calculated using:

Curative/preventive ratio = (Control ulcer index) – (Test ulcer index) X 100 / (Control ulcer index) (Darbar et al., 2013) was -47 and -32 for groups 4 and 5.

4.1.1 Discussion

Acid secretion

The experimental results obtained showed the plant extract exhibited anti secretory activity on gastric acid at lower doses (25mg/kg) significantly which declined gradually with dosage increase. This was may be as a result of the presence of lectins in the plant (Blitz et al., 1963) which are proteins/glycoprotein capable of recognizing and binding to

carbohydrate moieties (Bardocz et al., 1995). These have also been known to inhibit aminopyrine uptake by parietal cells (Healey et al., 1998).

However, fall in the inhibitory action on mucosal acid secretion (starting with 50mg) was seen gradually becoming constant (with 100mg and 200mg). This appeared to be due to the presence of increasing doses of salicylic acid (Blitz et al., 1963), with every subsequent dose of aloe vera extract administered. Later on the rate of antagonist action of salicylic acid seemed to be counter balanced by the increased amount of therapeutic acid inhibitory components at higher doses (100mg and 200mg). Salicylic acid inhibits cycloxygenase enzymes (COX-1and COX-2) which is the toxicity of this compound and all NSAIDs. (Fauci et al .,2008) This manifests as gastrointestinal mucosal ulceration and renal dysfunction. COX-1 (expressed the stomach platelets, kideys, and endothelial cells) isoform is key in the synthesis of prostaglandin are derived from esterification of arachidonic acid formed from phospholipids (cell membrane) by the action of phospholipase A_2. Prostaglandins play a crucial role in gastric epithelial defense/repair by regulating the release of mucosal bicarbonate and mucus. They inhibit parietal cell secretion, and maintaining mucosal blood flow and epithelial cell restitution. This therefore results into susceptibility to injury by gastric acid accumulation. (Fauci et al 2008; Hollander, 1994; Wallace et al., 1995; Linder et al., 2000; Yusuf et al., 2004). Salicylic acid also causes mucosal injury by directly inhibiting mucosal cell proliferation (Levi et al., 1990; Yusuf et al., 2004). This anti-ulcer effect of *Aloe vera* (Hennessee and Cook, 1994) may have been due to its acid secretory reduction property, as is conventional anti-ulcer drugs (Ranitidine) (Muller et al., 1983; Sewing et al., 1983; Barr et al., 1983; Rabon and Michael, 1990; Yusuf et al., 2004). The microvascular system within the gastric submucosal layer is important in the subepithelial defense/repair system, providing HCO_3^-, which neutralizes the acid generated by parietal cell. (Fauci et al 2008) This microcirculatory bed provides an adequate supply of micronutrients and oxygen while removing toxic metabolic by-products (Fauci et al 2008). Ischemia by ligation of the celiac artery caused severe arterial insufficiency which led to a reduction of the gastric blood supply and subsequent weakening in the gastric mucosal defenses against the gastric acid. The reduction in blood perfusion caused accumulation of

hydrogen ions and increased back-diffusion through the ischemic mucosa (Becker et al., 2005) with oedema, hemorrhage and focal sloughing of the mucosa. (Haruna et al., 2012).

On the other hand the acid secretory rate was slightly more inhibited by the combination of *aloe vera* extract than the *aloe vera* extract administered with small doses (25mg) probably due to synergy between the two drugs given together against aloe vera given the fact that this is the most effective dose. *Aloe vera* worked via the already discussed mechanisms and ranitidine worked by the binding and blocking the action of histamine at its H2 receptors of the mucosal epithelium (Katzungu, 2009).

However the lower / short lived increasemental activity seen may be a result of antagonism by increasing levels of salicylic acid (Blitz et al., 1963; Yusuf et al., 2004) and its subsequent effects on the mucosal barrier causing leaking of higher levels of Hydrogen ions into the canaliculi and the vicinity of the stomach. (Guyton and Hall, 2006) The resultant sharp drastic fall in activity at 200mg dose of *aloe vera*- ranitidine combination was in effect further confirming the antagonistic effects of salicylic acid, now expected to be at peak concentration whilst the wanted therapeutic action of aloe vera and ranitidine was masked by this overwhelming mucosa impairment in the presence of mucosa ischemia thus augmenting the excess acid secretory situation. (Craig et al.,NY)

Incomparision with AV-IR(G4) and AVRan-IR(G5) groups the ranitidine (Ran-IR) group had the most effective inhibitory effect of gastric acid secretion through action on the H2 receptor causing inhibition of histamine mediated acid secretion from the parietal cells. The effect is much profound than of the AVRan-IR group probably because of lack of the antagonist effect of salicylic acid as seen in the combination.

The normal saline given with ligation /ischemic induction had a slight an ulcer action probably because of the direct dilution effect on the accumulating free radicals and thus buffering the pH of blood while at the same time normal saline increases the volume of plasma thus increasing perfusion of the stomach tissues effectively via other smaller mesenteric vessels and following reperfusion therefore, this providing easy transport and elimination of byproducts of metabolism.

Normal saline administered without ischemic induction had an expected high gastric acid level following starvation and anticipation of food due to rattling of laboratory equipments and sounds of people that could together have been a conditioned reflux stimulus for gastric secretion since they almost only get visited by attendants routinely to clean up and feed them. This acid secretory process is known as the cephalic phase, triggered by the sight, smell, taste or thought of food. The vagal response that resulted was mediated by acetylcholine via M3 receptors which yielded sensitization of Calcium channels thus causing influx of extracellular Ca^{2+} ions that ultimately act as second messengers causing acid production via regulation of enzyme action (H^+-K ATPase). (Guyton and Hall, 2006). All this in effect due to lack of a drug influence as previously seen.

Cytoprotection action

There was clear evidence of cytoprotection against ischemia induced ulceration especially at increasing dose more so when in the combination therapy situation. However at higher doses diminishing returns of the protective effect began un folding this probably was due to the antagonistic effect of an aspirin like compound in the extract. These were more prominent with the combination regimen.

For the AV-IR and AVRan-IR groups response was dose dependent. *Aloe vera* appeared to confer its cytoprotective effect via stimulation of macrophage and fibroblast activity, increasing collagen and proteoglycan synthesis, up regulation of phagocytosis and fungicidal activity of macrophages, promotion of the stability of growth factors, stimulation of granulation tissue, anti-inflammatory effect of plant sterols through bradikinase activation, Inhibitory effect on release of reactive oxygen species from human neutrophils via reduction of intracellular free calcium levels, Increasing angiogenic activity in endothelial cells extra. (Grundmann, 2012)

The anti ulcer effect of ranitidine was via its acid inhibitor effect.

4.1.2 Summary

Numerous peptic ulcer lesions were formed as a result of ligation of the celiac artery which consequently led to ischemia of the mucosa epithelium due to the occlusion of the microvascular circulatory system. Some of these lesions had prominent blood vessels and evidence of bleeding of the ulcers which appeared to have been caused by accumulation of free radicals and by products of metabolism. These effects were later cleared following reperfusion of the mucosa by releasing the binding on the celiac artery hence restoring blood supply. The effect of orally administered aloe vera phytoextract in reducing susceptibility to damage by these factors was remarkable being effective when administered in lower doses of 25mg (acid secretion; less effective towards cytoprotection). Conversely higher doses (50mg, 100mg and 200mg) were much more effective toward cytoprotection than acid secretion though less was observed with 200mg may be due to the synthetic inhibition of prostaglandin by higher concentrations of salicylic acid at this dose. Ranitidine an H2 histamine antagonist was very much effective in both cytoprotection and towards acid secretion. This was mainly seen with mono therapy of histamine rather than the combination mainly because of the antagonist effect of salicylic acid in the combination. Negative controls of NS plus ligation and NS minus ligation yielded results similar to those of ranitidine mono therapy and low dose *aloe vera* mono therapy respectively due to the plasma volume expansion element and dilution buffering of normal saline against H^+ ions and free radicals for the later while for the former was due to lack any inhibitory influence of any drug against acid secretion following a conditioned reflux stimulation in anticipation of food being given.

4.1.3 Conclusions

Aloe vera extract was effective in preventing acute ischemic gastric ulcers though less effective at higher doses.

Aloe vera ranitidine combination had a synergnistic effect on the prevention gastric ulceration due to acid and free radicals accumulation but was less effective at high doses.

There is an antagonistic effect due to accumulation of salicylic acid at higher concentration of the extract thus impending the desired cytoprotective effect and acid inhibitory effect.

Salicylic acid in *aloe vera* extract caused the acute toxic effect of the extract.

Normal saline had a slight effect in the prevention of acid secretion following ischemia.

4.1.4 Recommendations

The research should be repeated using a different anti histamine and a proton pump inhibitor as positive control

The research should be repeated using aloe vera phytoextracts extracted by a different method other than one in this research.

More research should be done in this area of ischemic reperfusion in order to compare results for conclusive evidence of aloe vera's benefit in the prevention of ulceration.

CHAPTER V

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5.1.2 APPENDICES/ ANNEXES

Table1. Table of control groups that related ulcer number, length, grade and index

	Ulcer Number	Ulcer Length (cm)	Average UL	Grade	Ulcer Index
GI(NS)				0	0
G2(NS-IR)	Many(>15)	0.1-0.4	0.2	2	30
G3(RanIR)	4	0.1	0.1	1	4

Table2. Table of test groups that related ulcer number, length, grade and index

Group (AV-IR)4	Ulcer Number	Ulcer Length (cm)	Average UL (cm)	Grade	Ulcer Index
A(25mg/kg)	2	0.2-0.4	0.3	2	4
B(50mg/kg)	6	0.0-0.2	0.2	2	12
C(100mg/kg)	9	0.2-0.3	0.3	2	18
D(200mg/kg)	4	0.3-0.4	0.4	2	16
Group(AVRan)5					
E(25mg/kg)	5	0.2-0.3	0.3	2	10
F(50mg/kg)	1	0.0-0.2	0.2	2	2
H(100mg/kg)	7	0.1-0.4	0.3	2	21
G(200mg/kg)	2	0.0-0.3	0.3	2	4

Table3. Table of control groups that related the volume of supernatant and hydrogen ion concentration

Control	Volume of Supernatant used(mls)	Hydrogen Ion concentration (mEq)
NS	0.02	37.5
NS-IR	0.2	3.75
Ran-IR	0.3	2.5

Table4. Table of test groups that related the volume of supernatant and hydrogen ion concentration

Group (AV-IR)	Average volume of supernatant used(mls)	Hydrogen ion concentration(mEq/L)
A(25mg/kg)	0.04	18.7
B(50mg/kg)	0.08	7.32
C(100mg/kg)	0.06	12.6
D(200g/kg)	0.06	12.6
Group (AVRan-IR)		
E(25mg/kg)	0.06	12.6
F(50mg/kg)	0.07	11.0
G(100mg/kg)	0.14	5.37
H(200mg/kg)	0.01	75.1

ACID BASE CALCULATIONS

Calculating Hydrogen ion concentrations (mEq)

Using formula mEq = mg x val / Rmm (where val = valency Rmm = Relative molecular mass)

Rmm of NaOH = 40

40g of NaOH =1mole

0.1g of NaOH = 1/40 x 0.1

= 0.0025moles

1000mls of NaOH contained = 0.0025moles

0.3ml of NaOH are contained = 0.0025/1000 x 0.3

= $\underline{7.5 \times 10^{-7}\text{moles}}$

$$NaOH_{(aq)} + HCl_{(aq)} \longrightarrow NaCl_{(aq)} + H_2O_{(L)}$$

Mole ratio 1:1

Meaning 7.5×10^{-7}moles of NaOH reacted with 7.5×10^{-7}moles

Forinstance using 0.06 mls of supernatant that react with 0.1M NaOH; The was done for other volumes above.

0.06ml of HCl contained = 7.5×10^{-7}moles

1000mls of HCl contained = $7.5 \times 10^{-7} \times 1000 / 0.06$

= 0.0125M (moles/L)

RMM of HCl = 35.5 + 1 = 36.5

1mole of HCl weighed = 36.5g

0.0125moles of HCl weighed = (36.5 x 0.0125/1)g

= 0.46 g/L

Changing to milligrams / litre

1g HCl weighed = 1000mg

0.46g HCl weighed = 1000 x 0.46 /1

= 460mg /L

Valence of HCl = 1

Using $mEq = \frac{mg \times Val}{RMM}$

mEq = 460 x 1 / 36.5 = 12.6mEq / L

Table 5. Table showing the number of animals and their weights

Number of animals	Weight
>200	6
180-200	20
160-180	4
150-180	3

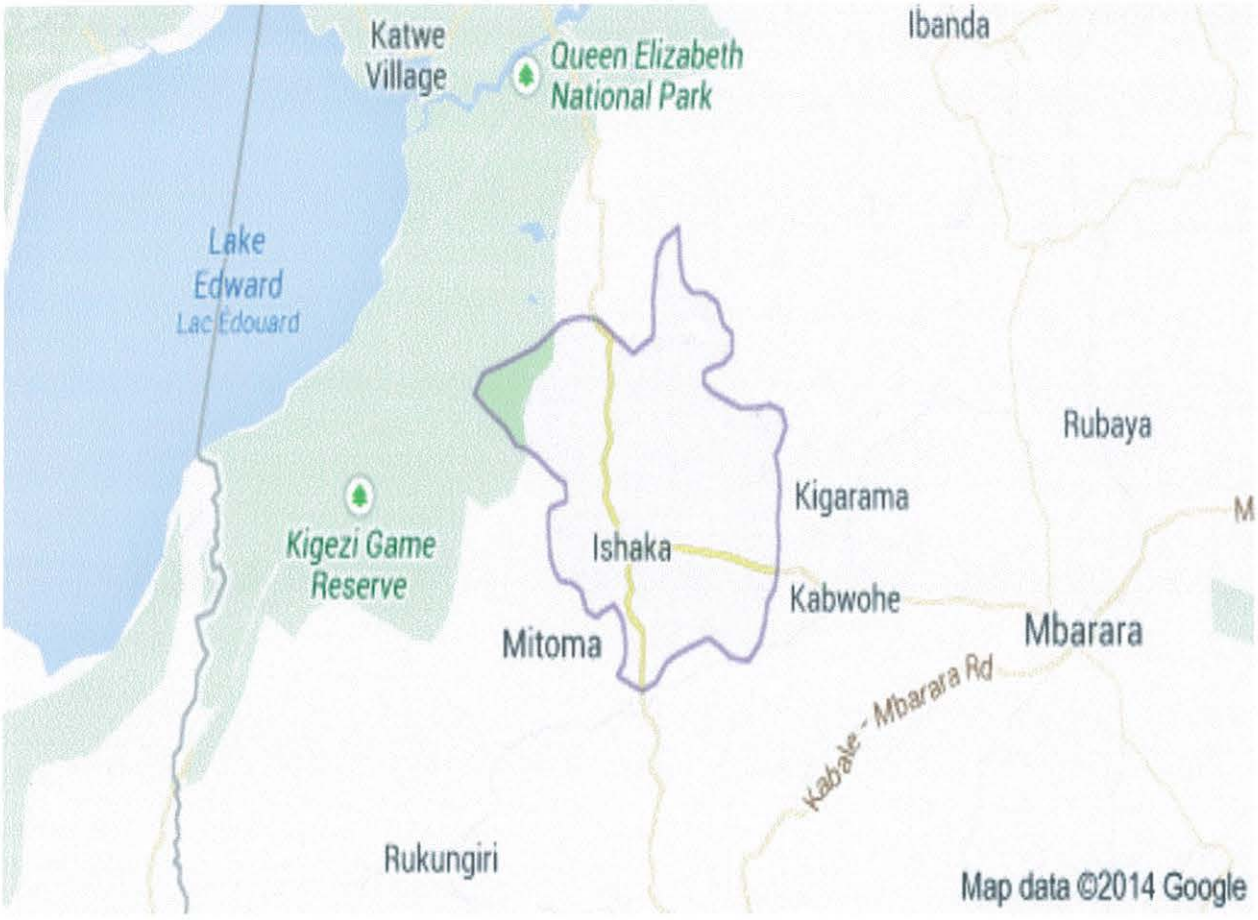
Table 6. Table showing the number of animals and their sex

Number of animals	Sex
12	F
21	M

Table7. Table that was used for animal dose selection at random (shows animals, and doses administered per animal.)

				Aloe vera doses			
Number	**Body marking**	**Body weight**	**Ranitidine dosage**	**25mg/kg**	**50mg/kg**	**100mg/kg**	**200mg/kg**
1	HD	211.2	0.17	0.1	0.2	0.4	0.8
2	TL	202.6	0.16	0.1	0.2	0.4	0.8
3	LA	188.7	0.15	0.09	0.19	0.37	0.75
4	LARA	194.7	0.16	0.097	0.19	0.38	0.77
5	RA	197.9	0.16	0.099	0.198	0.39	0.79
6	RL	185.0	0.15	0.09	0.185	0.37	0.74
7	LL	187.3	0.15	0.09	0.187	0.37	0.75
8	RLLL	187.2	0.15	0.09	0.187	0.37	0.75
9	HDTL	188.5	0.15	0.09	0.189	0.37	0.75
10	HDRA	182.3	0.15	0.09	0.182	0.36	0.72
11	HDLA	184.1	0.15	0.09	0.184	0.36	0.73
12	RALL	181.9	0.15	0.091	0.182	0.36	0.72
13	HDRL	186.6	0.15	0.093	0.186	0.37	0.75
14	HDLL	185.2	0.15	0.093	0.185	0.37	0.74
15	TLRA	197.7	0.16	0.099	0.198	0.39	0.79
16	TLLA	181.8	0.15	0.091	0.182	0.36	0.72
17	TLRL	203.7	0.16	0.102	0.204	0.4	0.8
18	TLLL	187.6	0.15	0.094	0.188	0.37	0.75
19	US	163.7	0.14	0.082	0.164	0.32	0.65
20	HDUS	174.0	0.14	0.087	0.174	0.34	0.69
21	RLLA	173.5	0.751	0.087	0.174	0.347	0.69
22	RALA	145.4	0.0727	0.073	0.145	0.291	0.58
23	RARL	154.2	0.771	0.077	0.154	0.291	0.62
24	LLLA	165.4	0.083	0.083	0.165	0.331	0.66

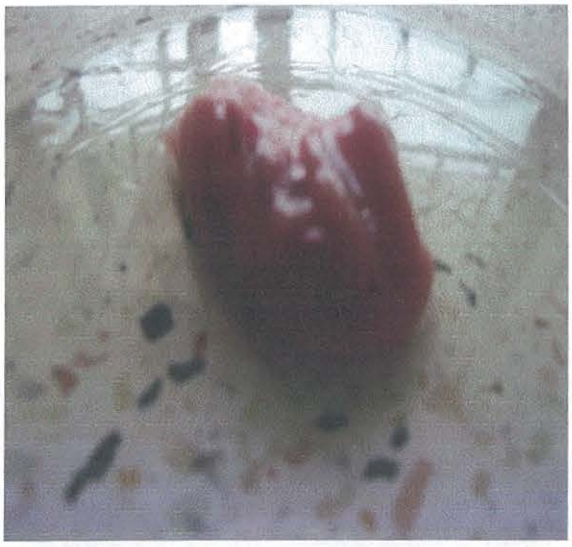
Map of Bushenyi district

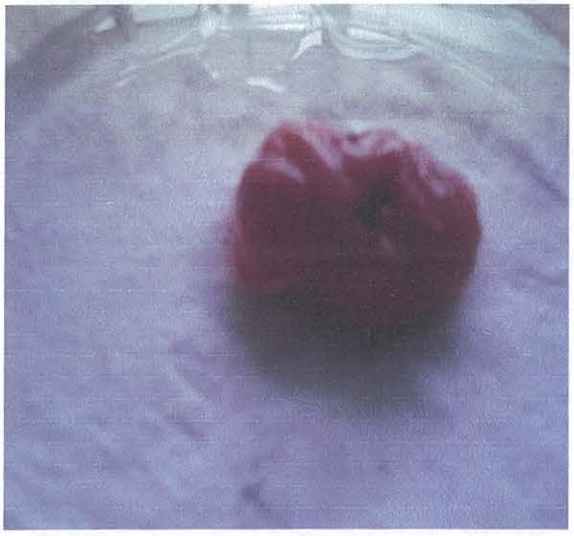
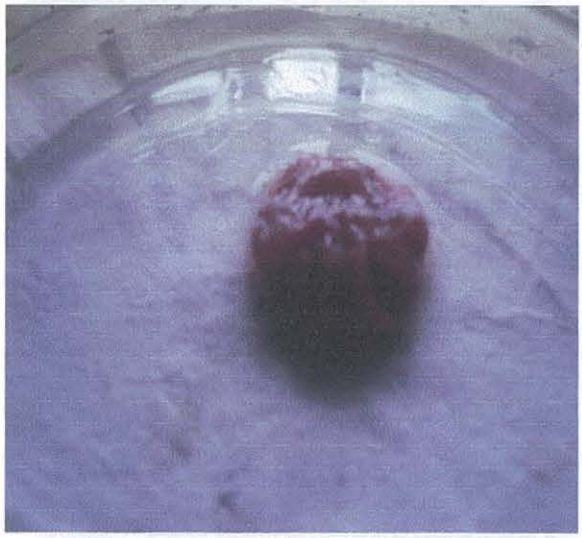
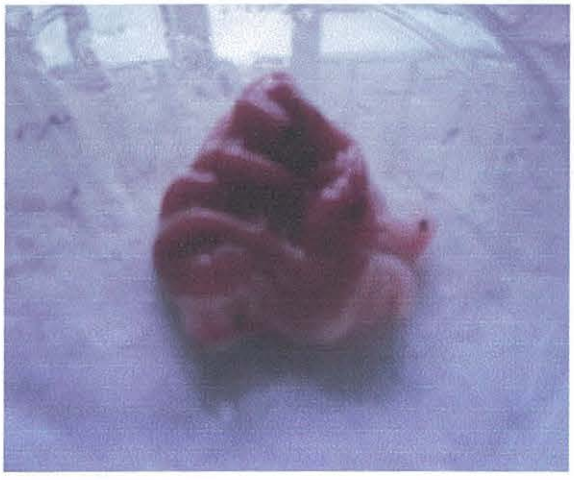
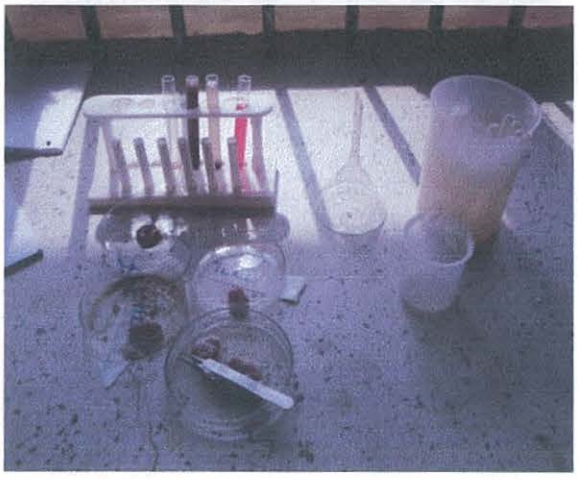


PICTURES

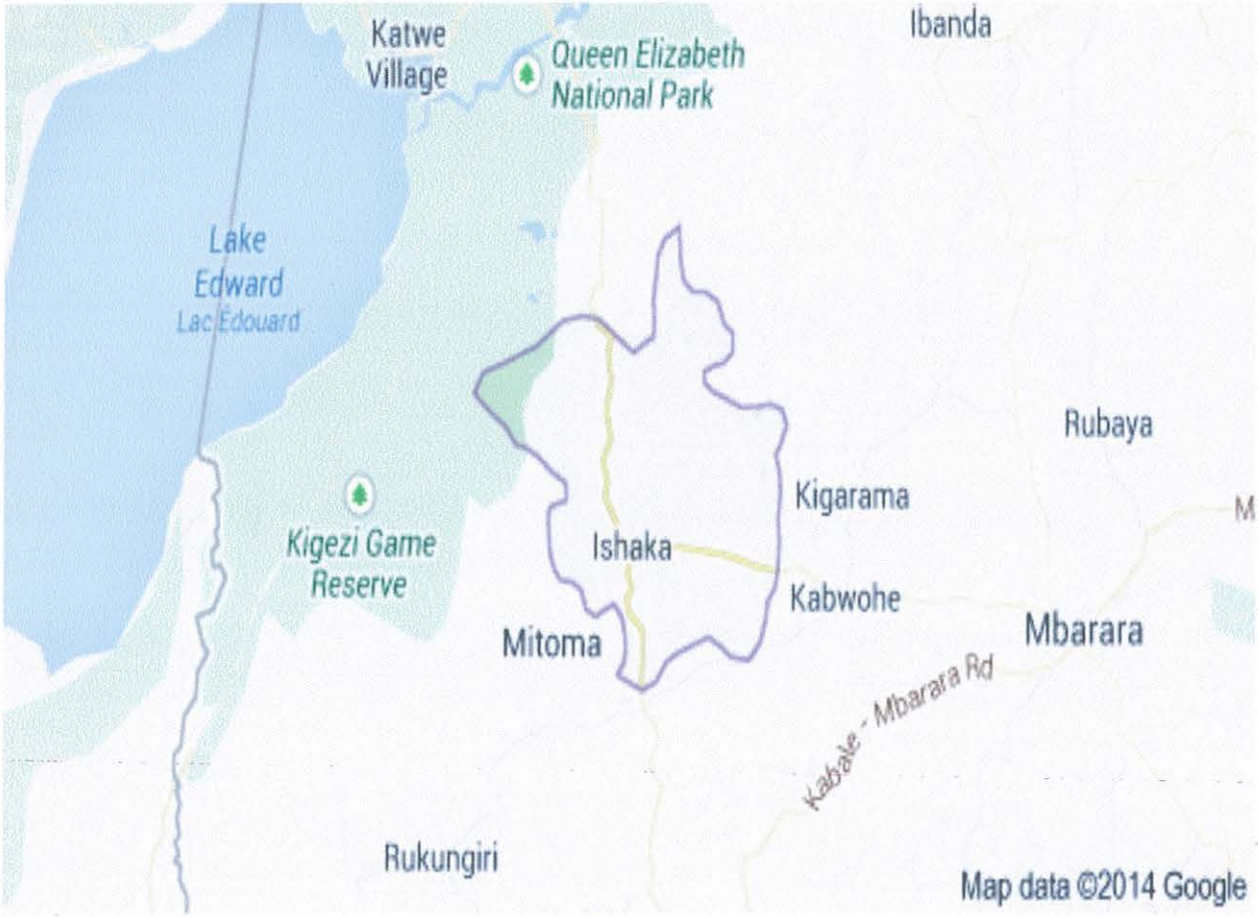
Photo I: Photos 1and2 (Tritration procedure

Photo II; Photos 4, 5, 6, 7, 8, 9 (Histopathalogical study)



Map of Bushenyi district



ANTIMICROBIAL SUSCEPTABILITY PROFILE OF MICROBIAL PATHOGENS AFFECTING WOUNDS IN PATIENTS ATTENDING SURGICAL WARD OF KIUTH.

CONDUCTED BY

KAGWISAGYE JOSEPH BPH/OOO1/91/DU

FEBRUARY 2014

ANTIMICROBIAL SUSCEPTABILITY PROFILE OF MICROBIAL PATHOGENS AFFECTING WOUNDS IN PATIENTS ATTENDING SURGICAL WARD OF KIUTH.

AN UNDERGRAUATE PROJECT
CONDUCTED BY

KAGWISAGYE JOSEPH BPH/OOO1/91/DU

SIGNATURE..KJs..... DATE...3rd/march/2014...

SUBMITTED TO
THE SCHOOL OF PHARMACY
KAMPALA INTERNATIONAL UNIVERSITY WESTERN CAMPUS
P.O BOX 71, BUSHENYI, UGANDA.

A REASERCH PROJECT SUBMITTED TO THE SCHOOL OF PHARMACY INPARTIAL FULLFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF PHARMACY

SUPERVISOR: MR.MANIGA JOSEPHAT
SIGNATURE..... DATE.....

FEBRUARY 2014.

DECLARATION :

I, Kagwisagye Joseph, BPH/ 0001/91/DU, a student of Pharmacy, school of Pharmacy, do declare that this project is original and all authors whose articles were quoted were appropriately cited and due permission were obtained before reproducing any article in this project.

Name: Kagwisagye Joseph

Registration Number: BPH/ 0001/91/DU

Sign/Date..KJS...../..3rd/march/2014.....

ATTESTATION

This is to confirm that this Undergraduate project ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF MICROBIAL PATHOGENS AFFECTING WOUNDS IN PATIENTS ATTENDING SURGICAL WARD AT KIUTH was conducted by Kagwisagye Joseph (BPH/ 0001/91/DU)

SUPERVISOR:

Mr. MANIGA JOSEPHAT

DATE.....sign.....

ACKNOWLEDGEMENT

I acknowledge my supervisor. Mr. Maniga J. for his contribution towards the success of this research project. My grateful thanks to the Dean of school of pharmacy Dr. Godwin O .

I also highly appreciate my sponsors for all the financial support they provided.

I would like to thank my beloved wife Madam Turyamwijuka Sylivia,entire family and friends for their support during the whole time of my study.

Finally to the almight GOD for seeing me through my studies.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

CDC - Center for Disease Control and prevention

CLSI - Clinical and Laboratory Standards Institute

KIU –WC - Kampala International University – Western Campus

KIUTH - Kampala International University Teaching Hospital

MHA - Mueller Hinton Agar

MIC - Minimum Inhibitory Concentration

MRSA - Methicillin Resistant *Staphylococcus aureus*

PRSA - Penicillin Resistant *Staphylococcus aureus*

QA - Quality Assurance

QC - Quality Control

VRE - Vancomycin Resistant *Enterococci*

AMC : - Augmentin

API: - Analytical Profile Index

CF[1] : - Cefaxone

CIP : - Ciprofloxacin

CLSI : - Clinical and Laboratory Standards Institute

GN : - Gentamycine

CRO[3] : - Ceftriaxone

E : - Erythromycine

Et : - Tetracycline

TX : -Cotrimaxazole

ABSTRACT

A cross section study was condcuted in KIUTH between November 2013 and January 2014 with the over all objective to identify the specific causal agents of wound infections and their sensitivity to commonly prescribed antimicrobial agents, and the specific objectives were to isolate and characterize the common microbial pathogens in wound specimens in KIUTH and to determine the anti microbial susceptibility of the microbial pathogens isolated from the wound specimens from KIUTH.

60 samples from wounds were taken to be analyzed in the laboratory using culture upon arrival in the laboratory. Cultures were done, followed by Gram stain and biochemical tests to identify pathogenic microorganisms. Culture media used were, MacConkey agar, Chocolate agar, Mannital salt agar, nutrient agar and fresh blood agar.

The study findings show that the most common type of microorganism isolated was the s.aureus 48.5%, followed by the Klebsiella 18.2%.then E.coli 16.2%, proteus and p.aerginosa represented 7.1% and 6.1% respectively, and the least grown microorganism was the s.epdidimis accounting for 4.0%.

This study concluded that there was predominance of Gram positive bacteria from the isolates. With *aureus* being the most common isolate. The study further showed most of the Gram negative and gram positives isolates were multiple resistant to commonly prescribed antimicrobial agents.

This study recommended that the hospital should perform routine culture and sensitivity and always use antimicrobial sensitivity test results to guide choice of antimicrobial agents, establish strict guidelines for antimicrobial prescriptions in treatment of microbial infections, limit the use of AMC and establish continuous surveillance to monitor antimicrobial susceptibility profile of the common isolates found in this study.

CHAPTER ONE

INTRODUCTION

Antimicrobials are often prescribed for the adjunctive treatment of skin and wounds infections. The choice of antimicrobial agent is usually based on previously published susceptibility testing and previous clinical success. There is concern that microbial pathogens have increased resistance to the currently prescribed antimicrobial agents (Vigil *et al.*, 1997). Susceptibility testing is the determination of the microbial profile of resistance to a number of antimicrobial agents. It would be ideal if susceptibility testing could always be undertaken before the prescription of antimicrobial agents. Unfortunately, it usually takes from several days to weeks to cultivate and do susceptibility tests on anaerobic bacteria (Riley, 2001).

Anaerobic bacteria are important because they dominate the diagnosed flora. They are commonly found in different infections. Some of these infections are serious and have high mortality rate (Summanen *et al.*, 1995). It has to be paid more attention to anaerobic infections because special precautions are needed for appropriate collection and transport of specimens. Isolation and identification of anaerobic bacteria can be complex, difficult, labor intensive, and expensive. The majority of these infections have caused mixtures of numerous strains of aerobic and anaerobic bacteria. Interpreting culture to establish the extent, to which any one particular anaerobe in the mixture is contributing to infection, is difficult (Brook *et al.*, 1997).

Treatment considerations for these mixed anaerobic infections are difficult and causing even more problem with increasing resistance among these groups of organisms. A number of antimicrobial agents have poor or no activity against some microbial pathogens (Wexler et al., 1998). Failure to provide

antimicrobial coverage against the anaerobes in a mixed aerobic-anaerobic infection may lead to inadequate response. This could, of course, be attributed to another factor such as the possibility of an untrained health personels (Holten *et al.*, 2000).The aim of the study is to study the sensitivity profile of microbial strains isolated from several types of infected skin and wounds toward several antimicrobial agents.

1.1 PROBLEM STATEMENT

Wound infections continue to be problematic in hospital settings and these infections are commonly caused by microbial pathogens (Pollack *et al.*, 2010). Microbial infections of the wounds remains a challenge to the hospitalized patients despite the improved multidisplinary approaches used to manage them in KIUTH and to the best of my knowledge, there is no data available regarding frequency of microbial wound infections and their antimicrobial sensitivity profile.

1.2 JUSTIFICATION

Infections of wounds are important cause of morbidity and mortality in patients undergoing surgery and add substantial financial burden and undue discomfort (Lichtenstern *et al.*, 2007). Treatment of microbial wound infections with antimicrobial agents is becoming a challenge for clinicians due to emergency and spread of multidrug resistance against antimicrobial agents. Therefore having knowledge regarding microbial population and their susceptibility profile is important in the management of wound infections. It is also crucial to take proper steps to restrain the spread of microbial infections within the surgical wards (Tahir, 1995).

1.3 Hypothesis

Wounds are caused by multiple microbial agents that are resistant to commonly used antimicrobial agents in KIUTH.

1.4 GENERAL OBJECTIVE.

To identify common microbial etiological agents affecting wounds and their antimicrobial susceptibility profile.

1.4.1 SPECIFIC OBJECTIVES

- To isolate and characterize microbial agents in wounds.
- To determine the antimicrobial susceptibility of the microbes isolated.

CHAPTER TWO

LITERATURE REVIEW

2.0 INTRODUCTION

Surgical wound infection is clinically defined as purulent discharge from the surgical wound (Bowler *et al*, 2001). Surgical wound infections are the second most common cause of nosocomial infections (Burker, 2003). There is a high rate of surgical wound infections and this is associated with higher mobidity, mortality and increased medical expenses (Rupp *et al*., 2003). In spite of the new antimicrobial agents available today, surgical wound infection still remains a threat due to secondary microbial contamination and widespread use of prophylactic antimicrobial agents that lead to emergence of multi-drug resistant microbes (Sohil *et al*., 2006). The risk of developing surgical wound infection depends on the number of microbes that colonise the surgical wound (Dohen *et al*., 2009). While the operating wound following surgery is considered to be "clean", the surgical wound may be contaminated by microbes (Kühme *et al*., 2007). Both aerobes and anaerobes bacterial pathogens infect wound Michael,1998), delaying the wound healing process (Bessa *et al*., 2013) Wounds are caused by multiple microbial agents that are resistant to commonly used antimicrobial agents in KIUTH. Microbial wound infections add suffering to patients resulting into disabling conditions that reduce the quality of life. Sheng *et al*., 2005). Because most microbial wound infections are multidrug resistant to antimicrobial agents and therapy to these infections are always difficult to manage(Schreckenberger *et al*., 2003), results into increased drug costs. More to that, such infection requires additional diagnostic tests Plowman, 1999).

2.1 MICROBIAL INFECTIONS OF WOUNDS

A wound is the result of physical disruption of the skin, one of the major obstacles to the establishment of infections by microbial pathogens in internal tissues. When microbes breach this barrier, infection can result (Bison *et al.*, 1996). The most common underlying event for all wounds is trauma. Trauma may be accidental or intentionally induced. The latter category includes hospital-acquired wounds, which can be grouped according to how they are acquired, such as surgically and by use of intravenous medical devices. Although not intentionally induced, hospital-acquired wounds can be the pressure sores caused by local ischemia, too. They are also referred as decubitus ulcers, and when such wounds become infected, they are often colonized by multiple microbial species (Janda *et al.*, 1997).

Wound colonization refers to non replicating microorganisms within the wound, while in infected wounds, replicating organisms exist and tissue is injured. All patients are colonized to some degree by extrinsic organisms, and the vast majority of these exist in either a mutualistic or commensal relationship with the host. Neither of this colonization is considered infectious. Non-pathogenic organisms can become pathogenic given specific conditions, and even the most virulent organism requires certain circumstances to cause a compromising infection. The variables involved in the outcome of a host becoming inoculated by a pathogen and the ultimate outcome include; the route of entry of the pathogen and the access to host, the intrinsic virulence of the particular organism, the quantity or load of the initial inoculants and the immune status of the host being colonized. It can be difficult to know which chronic wounds are infected (Reddy *et al.*, 2012).

The microbial pathogens that cause wound infections include staphylococcus, pseudomonas, Klebsiella, proteus species, Escherichia coli, clostridium and bacteroides species (Shittu *et al.*, 2002). Most wounds have polymicrobial populations and majority of the genera are antimicrobial resistant (Otokuneto *et al.*, 1990).

Most wound infections can be classified into two major categories: skin and soft tissue infections, although they often overlap as a consequence of disease progression (Khan *et al.*, 1993). Infections of hospital-acquired wounds are among the leading nosocomial causes of morbidity and increasing medical expense. Routine surveillance for hospital-acquired wound infections is recommended by both the Centers for Disease Control and Prevention (Haley *et al.*, 1985) and the Surgical Infection Society (Condon *et al.*, 1988).Staphylococcus aureus, coagulase negative staphylococci, Enterococcus spp., and Escherichia coli are the most frequently isolated pathogens from surgical wounds (Howard *et al.*, 1995). Most of the wound infections caused by antimicrobial-resistant pathogens, such as methicillin-resistant S. aureus (MRSA), or by Candida albicans are associated with increasing numbers of severely ill and immunocompromised surgical patients and the impact of widespread use of broadspectrum antimicrobial agents. Some of the surgical woundare caused by unusual pathogens, such as Clostridium perfringens, Rhodococcus bronchialis, Nocardia farcinica, Legionella pneumophila and Legionella dumoffii, and Pseudomonas multivorans and these have been associated with contaminated adhesive dressings, elastic bandages, colonized surgical personnel, tap water, or contaminated disinfectant solutions (CDC, 1992).

1.3 SUSCEPTABILITY OF MICROBES INFECTING WOUNDS

Although the primary purpose of antibiotics is to treat infection, prophylaxis associated with surgical practice accounts for up to half of all antimicrobials prescribed. Most uncomplicated surgical or traumatic wounds heal normally without the need for prophylactic antimicrobial treatment, although the involvement of foreign materials such as sutures, dirt, grafts, or prosthetic devices may increase the risk of infection in clean wounds (Periti *et al.*, 1998). However, in surgical wounds that are potentially heavily contaminated with endogenous aerobic and anaerobic bacteria derived from the disruption of mucosal surfaces (e.g., by gastrointestinal and gynecological surgery) or in severe traumatic wounds that are heavily contaminated with exogenous microorganisms, antimicrobial prophylaxis is effective in reducing infection and is recommended as a routine procedure. Surgical infections are frequently polymicrobial,

and the role of both aerobic and anaerobic bacteria in the pathogenesis of these infections is well recognized (Rotstein, 1998). The choice of prophylactic antimicrobial agents should cover both facultative and anaerobic intestinal bacteria to achieve high success rate . In established mixed infections, failure to target both facultative and anaerobic bacteria often leads to a poor clinical outcome (Gorbach, 1994). Combination therapy with an aminoglycoside (e.g., gentamicin) or a cephalosporin (e.g., cefuroxime or cefotaxime) plus clindamycin or metronidazole has proved to be very effective. Particularly in contaminated surgery, where excessive populations of gram-negative bacteria are likely to be present, careful selection of antimicrobial agents is required since some are known to influence endotoxin liberation and hence septic shock (Periti *et al.*, 1998).

The majority of chronic wounds (e.g., leg ulcers, foot ulcers, and pressure ulcers) are characterized by a polymicrobial aerobic-anaerobic microflora. Consequently, the careful use of broad-spectrum antimicrobial agents is likely to be the most successful treatment in the management of clinically infected chronic wounds. The presence of anaerobic bacteria in foot ulcers of diabetic patients has been associated with a greater likelihood of the patient becoming febrile, developing a more serious deep-wound infection, and requiring amputation (Goldstein, 1994). Since leg ulcers and foot ulcers often exhibit a similar microflora to pressure ulcers, such advice could probably be extended to cover a wider variety of chronic wound types.

Antimicrobial resistance is higher among gram negative isolates as compared to the gram positive bacteria. However, studies have shown that most gram negatives enteric organisms affecting wounds are sensitive to Amoxycillin-clavulanate and Ceftazidime but some like Klebsiella spp are highly resistant Sonawane *et al.*, 2010). Such multidrug resistance is a common problem in hospital pathogens such in addition to the later, *Proteus* spp. *Staphylococcus aureus*, *E. coli*, *Pseudomonas* spp. (Adegoke *et al.*, 2010). Most isolates from wounds are resistant to cephalosporin, quinolones and aminoglycosides, an

implication of empirical therapy which is a growing problem in medical practice today (Sonawane *et al.*, 2010).

2.4 ETHICAL CONSIDERATIONS.

Informed consent on this investigation was sought and obtained verbally from each subject and in writing from the Institutional Research Ethics Committee (IREC) of KIU-WC, before commencement of this study. A letter of introduction to permit the research to be carried out was issued by the Hospital management. Patients that cannot read or write in English language and give their verbal approval to the aims and benefits of this investigation were explained to them in their local language.

CHAPTER THREE

MATERIALS AND METHODS

3.0 STUDY LOCATION AND STUDY DESIGN

The study was carried out at Kampala International University Teaching Hospital (KIUTH – Western campus). This hospital is located in Ishaka, Bushenyi District, along Mbarara – Kasese road, approximately 56km from Mbarara. It has bed capacity of approximately 300, though it has small admission number and occupancy. The study was prospective cross sectional in nature involving collection wound pus from patients of all ages and sexes admitted in the mentioned hospital, meeting the exclusion and inclusion criterion.

3.1 SAMPLE SIZE

Sample size (S) was determined using a calculation using t the formula below Robert v. Krejcie(1970)

$= X2NP\ (1- P) \div d2\ (N-1) + X2P\ (1- P).$

= *required samples*

\2 = the table value of chi-square for 1 degree of freedom at the desired confidence level

3.841).

V = the population size. =1000

? = the population proportion (assumed to be .50 since this would provide the maximum

Sample size). =0.2

l = the degree of accuracy expressed as a proportion (.05)

60 samples were involved in the study.

The prevalence of wounds infections is at 28.7% in Mulago National Referal Hospital (J.Ojulong et al, 009)

3.2 EXCLUSION AND INCLUSION CRITERIA

The study population included only patients admitted in the surgical ward and has a wound. Those patients without any wound were not included in the study. Also outpatients did not participate in the study.

3.3 COLLECTION OF SAMPLES

Specimen collection, packaging and transportation, was carried out according to Uganda's Ministry of health standard operating procedures (CPHL-2009). The specimens were collected aseptically using sterile swabs (Shiny Medical Disposable Co., Ltd). Swab specimens were taken from deeper sites in order to capture anaerobes and two swabs from each patient.

3.4 CULTIVATION OF MICROORGANISMS

MacConkey agar (MAC), Blood agar base (BA), Chocolate agar (CHOC) and Mueller Hinton(MHA) for sensitivity testing agar medium were prepared according to manufactures (Mast Group Limited UK) nstructions. They were sterilized at 121^{0}C for 15 minutes holding time in an autoclave. Ten percent 10%) blood agar was prepared by mixing 10 ml fresh sheep blood with 90 ml molten blood agar base at ibout 45^{0}C. About 20 ml of each medium was dispensed on a sterile disposable plastic petri dish and llowed to set.

Wound specimen (pus or pus swab) were inoculated onto Chocolate agar with 5% sheep BA and MAC Mast Group Ltd., Merseyside, UK), using a calibrated wire loop (1/500ml) and incubated aerobically at 5^0- 37^{0}C and another plate of CHOC with 5% sheep blood agar was inoculated with wound specimen nd incubated anearobicaly for 18 - 24 hours after which the plates were observed for growth. The ifferent colonies were sub-cultured onto new plate for purity plates. A smear was made after swab noculation and stained with gram stain then examined microscopically for preliminary identification of athogens.

3.5 EVALUATION OF CULTURE

Staphylococcus was identified using the following tests; Gram stain, catalase, and both slide and tube coagulase tests (Forbes *et al.*, 2007). Streptococcal bacteria was identified by observing haemolysis on BA, Gram stain, catalase ((Forbes *et al.*, 2007), CAMP (Christie, Atkins, Munch, Peterson) test (Christie *et al.*, 1944) and Bile Esculin hydrolysis test for differentiation from enterococcus(MacFaddin,1980). Gram stain and gelatin stab (Murray *et al.*, 2003) were used to identify Bacillus species. Diptheroids was identified using Gram reaction, catalase, oxidase, Urease and carbohydrate fermentation (glucose, starch, maltose, sucrose) with acid fermentation (Forbes *et al.*, 2007). API 20E and API 20NE (bioMerieux^{R}, France) was used to identify gram negative bacteria.

3.6 ANTIMICROBIAL SENSITIVITY TESTING

Antimicrobial susceptibility test was carried out using sterile MHA(Mast Group Ltd., Merseyside, UK). The sterile MHA plates were kept at $2^{O}C$ – $8^{O}C$ refrigeration and brought to room temperature before use.

Bacterial isolates were brought to a uniform density by aseptically diluting 24 hour colonies grown on Nutrient Agar (purity plate) in 5 ml bioMérieux sterile 0.85% saline ampules until they matched McFarland Standard 0.5 (purchased as part of bioMérieux McFarland Standard set. MHA plates were inoculated using sterile cotton swabs in the confluent pattern as in the Kirby-Bauer procedure (Jorgensen et al., 2007).

Commercially prepared antibiotic disc (Mast Group Limited UK) were used. Kirby-Bauer's CLSI, 2007 modified disc diffusion technique for the antimicrobial susceptibility test was adopted in this investigation. After incubation at 35- $37^{0}C$ for 18-24 hours, zone sizes were measured and interpreted according to CLSI, 2007. The criterion for antimicrobial inclusion was based on first line broad

spectrum antimicrobial agents commonly used in Bushenyi and in particular KIUTH following guideline for antimicrobial agent selection during susceptibility testing by CLSI, 2007. The antimicrobial discs that were used and their disc antibiotic contents were Ciprofloxacin (5µg), Gentamicin (10µg), Augmentin (10µg), Cotrimoxazole (300µg), Oxacillin (1µg), Ceftriaxone (30µg), Cefuroxime (30µg), Erythromycin (15µg), Imipenem (10µg), Vancomycin (30µg), Clindamycin (2µg), and Tetracycline (30µg).

3.6.1 ANTIMICROBIAL SENSITIVITY RESULTS:

Zones of inhibition were read and compared with the values of susceptibility interpretive breakpoints issued by the CLSI - 2007 (Wiin et al., 2006) to determine the degree of sensitivity to each antimicrobial agent tested on each strain isolated, following 18 hours of incubation at 35- 37°C on MHA, plated 4 mm deep on 100 or 150 mm agar plates, following the Kirby-Bauer test protocol (Jorgensen et al., 2007), using aseptic techniques. Zones of inhibition were measured using a transparent ruler. A positive control of inoculation procedure, with no antimicrobial discs applied was used for each round of testing to verify proper inoculation protocol. Control strains obtained from CPHL – Kampala Uganda, suggested for each vial of antibiotic discs by Mast Group Ltd., Merseyside, UK manufacturer, (50 discs per vial) were used to verify performance of antimicrobial discs.

3.7 DISPOSAL OF LABORATORY WASTE

Laboratory wastes generated throughout the research process were safely processed and decontaminated through segregation preferably into colour coded discard containers according to methods; disposal and associated hazard i.e. Infectious wastes were disposed and transported into red bin. Personal Protective gears were considered when handling wastes. Biohazard waste like cultures, plates, media and other liquid or solid materials that contain or come in contact with living cells, pus and pus swabs as well as urine containers were sterilized by autoclaving at121^{0}C for 15 minutes prior to disposal. Working bench

surface was decontaminated with Sodium hypochlorite solutions containing 5 g/l available chlorine, which is recommended for dealing with Biohazardous spillage and which might contain large amounts of organic matter (Bloch S 2001).

3.8 DATA ANALYSIS

Data collected was entered into the excel data sheet, and then exported to Statistical Package for Social Science (SPSS) for analysis and presented using tables.

CHAPTER FOUR: STUDY FINDINGS

4.1 DEMOGRAPHIC INFORMATION

A total of 160 patients were involved in this study, majority of these patients were males 59.4% (95) and females were 40.6 % (65). Patients were grouped into 8 different age groups, according to table 1 below, most of the samples were taken from patients within the age group of 10-19(53%), followed by 20-29 who were 30(30%). 30-39 were 25(25%), 1-9 were 21(13.1%), 40-49 were 16(10%), 50-59 were 8(5%), 60-69 were 4(2%).The least number of samples came from patients within the age group of 70-79, 3 samples representing 1.8%.)

Age groups of the patients									
AGE.		1-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	Total
Sex	Male		36	14	14	8	7	1	2	95
	Female		17	16	11	8	1	3	1	65
Total			53	30	25	16	8	4	3	160

Table 1: Cross tabulation showing distribution of gender and age groups

Table 1 above shows that most of male patients were in the age group of 10-19 and these were 36, followed by 20-29 and 30-39 which each had 14 cases, 40-49 and 50-59 were 8 and 7 respectively,in age group of 60-69 there were only 1 patients and 2 patients in 70-79.

Most of the females were in the age group of 10-19 (n=17) as shown by table one above,followed by 20-29 age group that had 16 patients , then 11 patients in age group of 30-39,in both age group of 1-9 and 40-49 there were 8 patients. the female patients in the age group of 60 -69 were 3.The least number of females were in the group of 70-79 and 50-59 which were each having one case.

4.2 DISTRIBUTIO ... MICROBES ACCORDING TO THE GENDER OF THE PATIENT.

Different microorg ...isms were isolated, they were 6 in number and these included; E.coli, Klebsiella, S.aures, S.epididmis, ...eroginosa, and Proteus.

Table 2 below shov ...hat the microbial agents isolated were more common in the male than the female. According to the ta ... of the 95 males male patients, 35 did not have the microbes while 60 were nfected by differen ...ecies of microbes representing 63% while of the 65 female patients, 26 did not nave any microbial i ...tion. therefore 39 were infected representing 60%.

	Gender	
Microbial isolates	Male	Female
E.coli	8	8
Klebsiella	10	8
S.aures	33	15
P.aeroginosa	4	2
S.epididmis	1	3
Proteus	4	3
No organism isolate...	35	26
TOTAL	95	65

Table 2: distribution ...icrobes by respondent's gender

According to table ... above. the most common type of microorganism growth was the S.aures 48.5%, followed by the Kl ...lla 18.2%,then E.coli 16.2%. Proteus and P.aeroginosa represented 7.1% and 5.1% respectively, a ...the least grown microorganism was the S.epididmis accounting for 4.0%.

4.3 SUSCEPTIBIL[illegible] PROFILE OF GRAM POSITIVE BACTERIA.

Drug	S.aureus (n=48)		S.epididmis (n=3)	
	R	S	R	S
TET(10µg)	42	6	3	0
OX(30µg)	19	29	1	2
CRO^3(30µg)	17	31	2	1
CIP(5µg)	28	20	0	3
SXT(25µg)	2	46	2	1
VA(30µg)	8	40	1	2
LEV(5µg)	12	36	2	1
AMC(30µg)	48	0	3	0

Table 1: Antimicrob[illegible]usceptibility for the isolated gram positive bacteria. S=sensitivity,R=resistance.

A number of differe[illegible]ntimicrobial agents were set for the 2 gram positive bacteria isolated that is S.aures and S.epidid[illegible]. the antimicrobial agents set were TET(10µg), OX(30µg), CRO^3(30µg), CIP(5µg), SXT(25µ[illegible]VA(30µg), LEV(5µg), and AMC(30µg).

All S.aures isolated[illegible]e resistant to AMC and, most of them were resistant to TET that is 42(87.5%) and only 6 were se[illegible]ive to TET, with OX the number of S.aureus that were sensitive were 29 and 19(39.6%) were resi[illegible]. The number of S.aures that were sensitive to CRO^3 that is 31 was greater than those that were resi[illegible]t that is 17(35.4%), with CIP 28(58.3%) were resistant and 20 were sensitive. However most of S.[illegible]s were sensitive to SXT and VA that is 46 and 40 respectively, the resistant were 2(4.2%) and 8(16.7[illegible]spectively. Of the isolated S.aures 36 were sensitive to LEV and 12(25%) were resistant.

All isolated Epididi[illegible]s were sensitive CIP and resistant to both TET and AMC, for both OX and VA the number of Epididim[illegible]at were sensitive were 2 and 1(33.3%) was resistant. With CRO^3, LEV and SXT

the same number of ididimis were sensitive and resistant that is 1 and 2(66.7%) respectively to these antimicrobial agents.

4.4 SUSCEPTIBILITY PROFILE OF GRAM NEGATIVE BACTERIA.

Drug	E.coli (n=16)		Klebsiella (n=18)		P.aeruginosae (n=6)		Proteus (n=7)	
	R	S	R	S	R	S	R	S
TET(10µg)	13	3	8	10	3	3	7	0
CN	14	2	12	6	1	5	3	4
AMC(30µg)	16	0	16	0	6	0	7	0
CIP(5 µg)	9	7	10	8	2	4	5	2
E	15	1	11	7	6	0	3	4
CRO^3(30µg)	15	1	12	6	4	2	6	1
CF^1	12	4	11	7	4	2, -	5	2

Table 5: Antimicrobi susceptibility for the isolated gram negative bacteria.R=resistance; S=sensitivity.

A total of 4 differen ram negative bacteria were isolated these were: E.coli, Klebsiella, P.aeroginosa, nd Proteus. Antimic bial agents set were 7 and these included: TET(10µg), CN, AMC(30µg), CIP(5 g), E, CRO^3(30µg) a d CF^1.

All isolated E.coli were re stant to AMC , with TET the number that were sensitive were 3 and 3(81.3%) were resist nt. The number of E.coli that were sensitive to CN were 2 and 14(87.5%) were esistant , for both E nd CRO^{3}the number that were resistant were 15 (93.8%) and 1 was sensitive. E.coli were sensitive t CIP nd CF^1 that is 7 and 4 respectively, the resistant were 9(56.3%) and 12 75%) respectively.

Klebsiella isolates were all resistant to AMC, for TET the number that were sensitive were 10 and (44%) were resistant. For both CN and CRO^3 the number of Klebsiella that were resistant were 12(67%)

and 6 were sensitive. The number that were sensitive to CIP were 8 and 10(56%) were resistant. Klebsiella were sensitive to F and CF^1 that is 7, the resistant were 11(61%).

None of P.aeroginosa isolated was sensitive to AMC and E , there were the same number of P.aeroginosa that were sensitive to TET as resistant, 3(50%). For both CF^1 and CRO^3 the number of P.aeroginosa that were resistant were 4(66.7%) and 2 were sensitive. P.aeroginosa isolates were sensitive to CIP and CN that is 4 and 5 respectively, the resistant were 2(33.3%) and 1(16.7%) respectively.

All isolates of Proteus were resistant to AMC and TET . For both CF^1 and CIP the number that were resistant were 5(71.4%) and 2 were sensitive. Noting that the number of Proteus that were sensitive to both CN and E were 4and 3(42.9%) were resistant. Only 1 Proteus isolate was sensitive to CRO^3 and the rest 6(85.7) were resistant.

CHAPTER FIVE

5.1 DISCUSSIONS

Table 1(a) findings shows that majority of specimens came in from the age groups of 10-19 (33.1%) of the total number of samples received in the laboratory. Followed by the 20-29 (18.8%) These age groups have been shown to show a lot of injuries resulting from many physical activities especially sports which they usually get traumatized.

The least group of people comes from the age group of 70-79 years (1.9%), the elders who mainly have chronic infections due to their compromised immunity; their cost of treatments are higher, the fear of prolonged hospital stay make some of these elderly to be abandoned at home without being brought to seek care in the wards.

The study findings show that the most common type of microorganism growth was the S.aures 48.5%, his agrees with the findings of the same study by other people in different regions, in east Africa Anvikar et al, 1999) , in India (Mitova et al, 2000,2009) and in Nigeria (Sule et al, 2002).

Klebsiella was the second most common isolated microorganism 18.2%. many factors are known to contribute towards the infections by this bacteria, in a recent review it was reported that the hands of health care workers and patient can play a significant role in the transfer of gram negative bacteria during cross infections (Gould, 1994).

E.coli isolated in 16.2%, Proteus and P.aeroginosa represented 7.1% and 6.1% respectively, and the least known microorganism was the S.epididmis accounting for 4.0%.

In this study it was observed that there is high rate of antimicrobial resistance to the antimicrobial agents that are commonly in KIUTH, a trend that is consistent with Moges F et al., 2002 and Mulu et *al.*, 2006 in Ethiopian Hospital. This remarkable high antimicrobial resistance could probably be due to ease of availability and indiscriminate use of these antimicrobial agents by clinician as indicated by Ibeawuchi et al (2002) in sub Saharan Africa and self medication by patients because there is always significant

associations between antimicrobial exposure and resistance (Harbarth *et al.*, 2001). Surprisingly, there is no record of antimicrobial prescription to patients based on laboratory susceptibility results, a system that can encourage antimicrobial resistance spread. For example Augmentin and tetracycline were the most ineffective antimicrobial agents, though these antimicrobial agents are continued to be used in this setting. However ciprofloxacin (quinilone) was the most effective antimicrobial agent for both gram negative and gram positive bacteria, a similar profile was observed by El Kholy *et al.*, 2003 in Egypt.

Most of the isolates in from KIUTH, were multiple drug resistant which could be an indication of plasmids playing an important role in the spread of drug resistant genes between these pathogens (Abula T *et al.*, 2004), and it is most likely that this could be due to increased chances of cross infection among in patients, directly or indirectly and circulating these resistant microbial strains (Harding G et *al.*, 1994) and probably due to excessive antimicrobial misuse (El Kholy *et al.*, 2003).

In this study, anaerobic bacteria were not isolated and this could have been due to the use of dry swab which might have hindered the isolation of anaerobic bacteria and the fact that majority of these organisms die in the presence of traces of oxygen and dry cotton swab has shown to be having. Other studies have also reported to have failed to isolate anaerobes for example a study conducted using 13,833 blood culture samples, of all, the 1855 microbial pathogens isolated , none was an anaerobic bacteria Moyo et al, 2010).

5.2 CONCLUSIONS

There was predominance of Gram positive bacteria from the isolates. With *S.aureus being* the most common isolate. Most of the Gram negative and gram positives isolates showed multiple resistances to commonly prescribed antimicrobial agents.

5.3 RECOMMENDATION

1. Perform routine culture and sensitivity test to guide choice of antimicrobial agents.

2. Establish strict guidelines for antimicrobial prescriptions in treatment of microbial infections.

3. Limit the use of AMC.

4.0 Establish continuous surveillance to monitor antimicrobial susceptibility profile of the common microbial isolates.

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APPENDICIES

WORK PLAN

	2013					2014	
Activity/Month	august	september	october	november	december	january	february
Proposal writing	XXX	XXX					
Data collection			XXX	XXX	XXX		
Data analysis					XXX		
Report writing					XXX		
Submission of report						XXX	XXX

BUDGET

No.	Item	Purpose	Cost
A	**Consumables**		
1	Culture media	Growth of microorganisms from wounds 500g	**400,000/=**
2	Biochemical testing kits and reagents	For identification of microorganisms	**1,000,000/=**
3	Antibiotic discs	For susceptibility testing	**800,000/=**
4	Stains and glass ware	To gram staining reaction and preparation of reagents	**100,000/=**
3	**Secretarial**		
1	Printing, photocopying and binding	Printing and binding of thesis	**100,000/=**
2	Internet services	Search for new information from the websites	**100,000/=**
3	**Transport**	To field to collect specimens	**150,000/=**
Total	**Two millions six hundred and fifty thousand**		**2,650,000/=**