Mechanisms of wound healing and gastro protective effects of ethanol leaf extract of *Jasminum Sambac* and *Hemigraphis Colorata* on HCL/ethanol-induced gastric injury in experimental animals

AHMED SALIM AHMED AL RASHDI

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Name of Candidate: Ahmed Salim Ahmed Al Rashdi

(New Passport No: 02844943)

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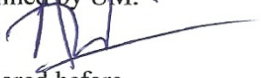
Candidate's Signature  **Date** 6/6/2013

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Designation: professor

Professor Dr Mahmood Ameen Abdulla
Lecturer
Department of Biomedical Science
Faculty of Medicine
University of Malaya

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ABSTRACT

Sprague Dawley rats weighing 225-250 g were used for evaluating wound healing and gastroprotective activities. The plant extracts of *J.sambac* and *H.colorata* were assessed *in-vivo* for toxicity study. Antioxidant properties of the crude extracts were determined using DPPH radical scavenging assay and total phenolic content. B-sitosterol and octyl gallate compounds were isolated from *J. sambac* and *H. Colorata* petroleum ether crude extracts by CC, TLC and HPLC and were evaluated for their gastroprotective activities.Macroscopic finding showed that *H.colorata* and *J.sambac* significantly accelerated wound healing compared to vehicle. Post wounding tissues collected on day 10^{th} for oxidative damage evaluation revealed significant increase in antioxidant enzymes (SOD and GPx) and decrease in the MDA level. Grossly, and histology showed smooth surfaces, marked reduction of edema and leucocytes infiltration of submucosal layer compared with ulcer group. Immunohistochemistry revealed overexpression of Hsp70 protein and downexpression of Bax protein in rats. Significant increase in the pH, mucus of gastric content and gastric PGE2, SOD, COX - 2 and reduced level of MDA were observed.

B-sitosterol compound showed significant gastroprotective activity against acidified ethanol-induced gastric damage in rats more than Octyl gallate compound. Moreover, β-sitosterol recorded significant increase in the pH, gastric mucus and decrease in the ulcer area. Biochemistry, histopathology and immunohistochemistry results revealed significant improvement in β-stosterol than Octyl gallate.Wound healing can be accelerated and gastric ulcer can be inhibited by the antioxidants activity of *H.colorata* and *J.sambac* leaves extracts and its active antioxidant ingradient β-sitosterol while the normal gastric status can be preserved.

Abstrak

Tujuan kajian ini adalah untuk menilai aktiviti perlindungan ekstrak etanolik bahagian daun*Jasminum sambac* dan *Hemigraphis colorata* terhadap dua jenis kecederaan tisu epitelium, luka dan ulser gastrik dan mekanisme potensi mereka. Tikus Sprague Dawley seberat 225-250 g telah digunakan untuk menilai penyembuhan luka dan aktiviti gastroprotective. Ekstrak tumbuhanJ.sambac dan H.colorata dinilai dalam ujian *in vivo* untuk kajian ketoksikan. Sifat antioksidanekstrak mentah telah ditentukan oleh menggunakan DPPH assay pemerangkap radikal; dan kandungan fenolik total (TPC). Sebatian b-sitosterol dan octyl gallate telah diisolasi daripada extrak mentah petroleum ether *J. sambac* dan *H. Colorata* denganmenggunakan kromatografi turus, kromatografi lapisan nipis TLC dan kromatografi cecairberprestasi tinggi (HPLC) serta penilaian untuk aktiviti gastroprotektif telah dilakukan.Untuk penilaian penyembuhan luka, kumpulan haiwan biasanya dirawat dengan 0.2 mlvehicle (getah akasia), gel intasit, 100 mg / ml dan 200 mg/ml bagi kedua-dua ekstrak tumbuhan.Penemuan makroskopik menunjukkan bahawa *H.colorata* dan *J.sambac* mempercepatkan penyembuhan luka berbanding vehicle. Analisis histologi terhadap penyembuhan luka menunjukkan lebar parut yang agak kurang pada penutupan luka, pengurangan inflamasi sel-sel, penambahan pemendapan kolagen dan proliferasi fibroblas oleh angiogenesis berbanding vehicle. Tisu cedera dikumpul pada hari ke-10 untuk penilaian kerosakan oksidatif mendedahkan peningkatan yang ketara dalam enzim antioksidan (SOD dan GPx) dan penurunan dalam tahap MDA. Untuk penilaian gastroprotective terhadap pengasidan etanol- penyebab ulser gastrik dalam tikus, tikus telah menerima pra rawatan secara oral dengan carboxymethylcellulose (CMC) sebagai kumpulan biasa, CMC sebagai kumpulan ulser, 20 mg / kg omeprazole sebagai kumpulan kawalan positif, 62,5, 125, 250,dan 500 mg / kg ekstak *J. sambac* dan 50, 100, 200, 300, 400 dan

500 mg /kg ekstak *H. colorata* sebagai kumpulan eksperimen. Sejam kemudian, CMC telah diberikan secara oral kepada kumpulan normal dan larutan berasid etanol telah diberikan secara oral kepada kawalan ulser, kawalan positif, dan kumpulan eksperimen. Tikus-tikus tersebut dikorbankan selepas sejam kemudian. PH gastrik, mukus dinding gastrik, kawasan ulser, histologi dan Immunohistokimia dinding gastrik telah dinilai. Ujian assay terhadap homogenat gastrik untuk menilai kandungan rostaglandin E2 (PGE2), cyclooxygenase II (Cox-II), SOD, dan MDA telah dilakukan. Kumpulan Ulser mempamerkan kecederaan mukosa ketara teruk berbanding yang dirawat dengan ekstrak yang mana menunjukkan perlindungan yang signifikan terhadap kecederaan mukosa gastrik seterusnya menyebakan pengurangan yang ketara di kawasan ulser. Histologi menunjukkan permukaan yang licin, menandakan pengurangan edema dan penyusupan leukosit lapisan submucosal berbanding dengan kumpulan ulser. Immunohistokimia mendedahkan overexpression protein Hsp70 dan downexpression protein Bax pada tikus. Peningkatan yang ketara dalam pH, mukus kandungan gastrik dan PGE2 gastrik, SOD, COX -2 dan tahap pengurangan MDA telah diperhatikan. Kompaun b-sitosterol menunjukkan aktiviti gastroprotective terhadap pegasidan etaholpunca kerosakan gastrik pada tikus, dan aktivitinya melebihi sebatian octyl gallate. Selain itu, β- sitosterol mencatatkan peningkatan yang ketara dalam pH, mukus perut dan pengurangan ulser di bahagian ulser. Keputusan ujian biokimia, histopatologi dan immunohistokimia menunjukkan keberkesanan β-stosterol adalah lebih baik daripada octyll- gallate.

Penyembuhan luka boleh dipercepatkan dan ulser gastrik boleh dihalang oleh aktiviti antioksidan eksrak daun *H.colorata* dan *J.sambac* dimana dengan kehadiran ramuan sebatian aktif β-Sitosterol, status gastrik yang normal dapat dicapai.

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations	Description
ALP	- Alkaline Phosphatase
ALT	- Alanine Transaminase
AST	- Aspartate Transaminase
BHA	- butylhydroxyanisole
BHT	- butylhydroxytoluene
CB	- Conjugated Bilirubin
CMC	- Carboxymethyl Cellulose
COX-1	- Cyclooxygenase 1
COX-2	- Cyclooxygenase 2
ddH2O	- double-distilled water
DMSO	- Dimethyl sulfoxide
DPPH	- 1,1-diphenyl-2- picrylhydrazyl
DW	- distilled water
FRAP	- Ferric Reducing Antioxidant Power Assay
GAE	- Gallic acid equivalent
HC	- *Hemigraphis Colorata*
HD	- high dose
H&E	- Hematoxylin and eosin

HPLC	- High performance liquid chromatography
JS	- *Jasminum Sambac*
LD	- low dose
LD50	- The median lethal dose of a substance
MRM	- Multiple reaction monitoring
NSAIDs	- Nonsteroidal-anti-inflammatory drugs
OMP	- Omeprazole
PGD2	- Prostaglandin D2
PGE2	- Prostaglandin E2
PGF2α	- Prostaglandin F2α (PGF2α) Receptor
PGI2	- Prostacyclin
PPIs	- proton-pump inhibitors
ROS	- Reactive Oxygen Species
SPSS	- Statistical Package Social Science
TBIL	- Total Bilirubin
TP	- Total Protein
TPC	- Total Phenolic Content
TPTZ	- 4,6-tri[2-pyridyl]-*s*-triazine
UA	- Ulcer Area
g	- gram
^{o}C	- celsius
hr	- hour
mg	- milligram
kg	- kilogram

Ml	- milliliter
w/v	- weight/volume
%	- percentage
µL	- microliter
µg	- microgram
mmoL	- millimol
L	- liter
Min	- minute
mm	- millimeter
µm	- micrometer
nm	- nanometer
rpm	- revolutions per minute
M	- molarity
kV	- kilovolt
V	- volt
Amu/s	- atomic mass unit
µmoL	- micromole
U/L	- unites/L
fL	- femtoliters
pg	- picogram

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Dedicating this thesis to my beloved

late Parents

God might keep them in Paradise

CHAPTER 1

INTRODUCTION

1.1 Background of the study

1.1.1 Wounds

Wound has been defined as the process of the disruption of normal architecture of a tissue. They may be closed (e.g. bruises, ruptures and sprains) or open as physical injuries that result in an opening or breaking of the skin (e.g. abrasions, avulsions, ballistic, hernias and excised or surgical wounds).(Enoch & Leaper, 2005) defined wound as a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, hematoma, laceration or an abrasion (Enoch & Leaper, 2005). There are several causes or factors, which may interfere with wound healing such as traumatic (mechanical, chemical, physical), intentional (surgery), ischemia (e.g. arterial leg ulcer) and pressure (e.g. pressure sore)(Branom, 2002).

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions (Muthusamy Senthil Kumar et al., 2006).Hence, for infection control, and for the restoration of disrupted anatomical continuity and disturbed functional status of the skin, appropriate method for healing of wounds is essential (Ayyanar & Ignacimuthu, 2009). Wound can be classified as being either acute or chronic and as partial thickness or full-thickness wounds. Acute wounds are defined as wounds that heal in a predictable and expected time. Chronic wounds are defined as wounds that are difficult to heal. This usually occurs in compromised patients who have an underlying pathology such as poor circulation or diabetes (Branom, 2002). Partial

thickness wounds involve the epidermis and may or may not involve the dermis. These wounds are shallow, moist, and painful. They mostly heal first with the initial inflammation response, then re-epithelialization. However, in full thickness wounds, healing begins after injury initiates a series of cellular and biochemical events that occur in coordinated and overlapping phases in the healthy host, which results in healing (Branom, 2002).

1.1.2 Wound Healing

Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of injury area and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue (Ayyanar & Ignacimuthu, 2009; Sumitra et al., 2005). Wound healing is a complex series of interrelated events that are mediated through the phases by a wide range of chemically co-ordinate cellular processes as well as hormonal influences (Chan et al., 2008). Chemical mediators such as growth factors, cytokines (small protein hormones that influence normal cell function) and chemokines (chemical mediators with a powerful chemotactic ability) combine to co-ordinate the healing process (Chan et al., 2008). These combine to return the damage tissue to as near normal function as possible, thereby allowing the body to repair injured tissue and restore skin integrity.

The process highly required the coordinated integration of cellular, physiological, biochemical events and molecular process, which result ultimately in connective tissue repair and the formation of fibrous scar (Hardy, 1989). Wound healing starts immediately after damage has occurred, but the mechanism and speed of healing, and the eventual nature of the regenerated tissue, depend on the type of wound.

However, the process depends on the types of tissue which has been damaged and the nature of the tissue disruption (Hardy, 1989). In wound healing, the wound environment change with the changing individual health status (Hardy, 1989).

Therefore this study is hoped to throw an insight on the wound healing activity of *J.Sambac* and *H.Colorata* especially in patients there by reducing the effect of amputation.

1.2. Peptic Ulcer

Usually Peptic ulcer occurs beside duodenum and stomach where can be observed in the gastric mucosa. Due to many changes in our life style make peptic ulcer amongst common diseases is associated with sore in stomach. Clinical symptoms and complications are as nausea, vomiting, severe epigastric sore, cardiac burning, losing eating desire (loss of appetite) (Ramakrishnan & Salinas, 2007a). The predominant reasons cause peptic ulcer is *H.pylori* and NSAID's intake.

It has been considered as common to cure ulcer is to avoid acidity related meals, avoiding consuming of ulcerogenic drinks and enough rest is strongly recommended. Over all, availability of certain medicines used to cure ulcer in a long term doses as PPI (Proton Pump Inhibitor) healing is promised(Vakil & Fennerty, 2003). Recurrent of disease, side effects of using various drugs leads to drug resistant and cure doesn't not reach to the level required entirely healing. Replasing has increased disease management cost whether in drugs cost and other exhibited needful (Alhaider et al., 2007).

Common medicinal plants used to treat different degrees of peptic ulcer and other related diseases with gastric sore. Currently, folk medicine is growing globally and

eastern world of developing countries considered herbs are the only choice of treatment.(Heinrich et al., 2004). Medicinal plants are the main source of primary and secondary active compounds such as alkaloid, flavenoid, terpinoid, tannins and others that involving direct or indirect to our health life(Falcão et al., 2008).Nowdays, pharmacological aspects on medicinal plants is attention of many scientists to investigate alternate medicines.

Many Malaysian plants used daily in our food as spice or flavor to give taste and preventive medicine. On the other hands, these plants are used traditionally to treat various diseases. Traditionally, hundreds of medicinal plants as well involved to boost and treat other human being ailments. Some such as *Phylanthus nirur, Melatoma malabathicum* (Hussain et al., 2008) suggested earlier contents of bioactive capacity to have cytogastroprotective properties in gastric mucosa. In this research project, basically on their used traditionally, two selected medicinal plants were used to study their cytogastroprotective properties in *vivo*. Some research studies have proved availability of toxic in few Malaysian plants in experimental animals and possible to human as well due to compounds activities. Toxicity evaluation of every medicinal plant is essential and vital procedure for any future commercial desire in product.

General practice physicians prescribe the current available medicines for peptic ulcer on suppressing and inhibition gastric acid secreted in gastric mucosa area as acid dependence provided by the sucralfate and bismuth, anti-muscalarins and omeprazole (Bighetti et al., 2005). The percentage of recurrence in ulcer patients is high despite the therapy on proton pump inhibition and H2 antagonist for a month to 6 weeks. However, the gastroduodenal ulcer healing is between 80~100% and recurrent within a year from the day of stopping management is about 40~80%(Miller & Faragher, 1986).

Despite, all these available drugs can cause aggressive reactions. Hence, more work is needed to seek for a safe, secured anti-ulcer and more effective (Ariyphisi et al.).

Several beneficial plant extracts are used for the inhibition of unwarranted gastric secretions, or to enhance mucosal defense mechanism, shift in proton pump, stabilizing surface epithelial cells or interfering with prostaglandin synthesis (Reyes-Trejo et al., 2008). Moreover, the plant extract are the important source for the new drug development due to more safety and high antioxidant composition.

Several medicinal plant extracts have been evaluated for enhancing mucosal defense metabolism, inhibition of undesired mucosal secretions, stabilized epithelial surface in order to enhance production of prostaglandin and to shift pump proton.(Reyes-Trejo et al., 2008).In addition, plants are better source of safe new drug discovery and rich of antioxidant capacity.

Therefore this study is hoped to throw an insight on the wound healing activity of *J.sambac* and *H.colorata*. Gastric is vital organ for food digestion and push out the chyme into duodenum for absorption process. The last step in digested chyme processed in gastrointestinal for absorption of essential elements, liquids and important nutrients. Normal metabolism takes place in healthy adult either at nutritional state, water level and other important elements might be interrupted by mild or sever ulcer(Sundaram et al., 2002).Gastro duodenal ulcer and wound becomes main reason for intestinal surgery which lead to increase mortality and morbidity. More in eastern world than western part of the world and varying prevalence's over the world(Yuan et al., 2006).Peptic ulcer is increasing within the population worldwide and scientists are expecting to involve significantly to improve awareness in ulcer, quality life of patients and economic system(Falcão et al., 2008). Moreover, nowadays brufen, and aspirin (NSAIDs) are

customs drug in many family which is aggressive tool to damage gastric mucosa which cause ulcer. Hence, researchers are looking for alternate medicine which safe and secured gastro protective and anti gastric ulcer to avoid any adverse reaction. Potential and promises medicinal plants, crude extracts and other form of natural products are widely used to cure diseases and mucosa ulcer damage(Al Mofleh et al., 2008).

1.3. Scope of study

This work focused on two of Malaysian medicinal plants, wide practiced in folk medicine. Plant crudes evaluated for wound healing and gastroprotective capacity.Ethanol was used to extract the selected plants. Evaluations of antioxidant power in crudes were performed by using DPPH and TPC. Furthermore, acute toxicity *in vivo* was evaluated to determine the safe dose which can be use in experimental animals. Overall, results of *Jasminum Sambac and Hemisgraphis Colorata* selected to be novel plants in wound healing and gastro-protective from necrotizing agent Hcl/ethanol induction model. Further study, was carried out to identify and isolated the active compounds. Bioactivity studies of the active compounds were evaluated *in vivo* to determine cytogastroprotective and possible mechanisms

1.4.1. General objectives

Wound healing activity and gastroprotective effect of ethanol leaf extract of *J.sambac* and *H.colorata* and their active compounds such *B-sitosterol* and *Octylgallate* against HCL/EtOH induced gastric ulcer in rats.Also, study on various markers in possible mechanism of *J.sambac* and *H.colorata* extracts and their active compounds.

1.4.2. Specific objectives

1- To determine the acute toxicity of *J. sambac* and *H. colorata* ethanol extracts in rats.

2- To determine the antioxidant properties of *J. sambac* and *H. colorata* ethanol extracts in *vitro*.

3- To study the wound healing activity of *J. sambac* and *H. colorata* ethanol extracts in normal rats grossly and histology.

4- To study the antiulcer activity of *J. sambac* and *H. colorata* ethanol extracts against HCl/Ethanol induced gastric ulcer in rats grossly and histology.

5- To assay the level of MDA as a marker of oxidative stress, the antioxidant enzyme SOD levels and protein concentration on homogenates from gastric mucosa and wound healing area.

6- To assay PGE2, COX-2 in the stomach tissue homogenate.

7- To investigate the Immunohistochemical markers (Bax and HSP-70) in the stomach tissues treated with *J. sambac* and *H. colorata* ethanol extracts.

8- To isolate and identify the active compounds β-sisterol and Ocytl gallate from *J.Sambac* and *H.Colorata* ethanol extracts respectively by CC, TLC, HPLC and LCMS.

CHAPTER II

LITERATURE REVIEW

2.1. Wound and wound healing

Smith defined wounds as common clinical entities in day-to-day life, which may be major or minor. He reported the process of wound healing to be classified into five phases, cellular phase (granulation), narrowing of wound area (wound contraction), collagen deposition (collagenation), epithelial covering (Epithelialisation) and scar remodeling (cicatrisation). He opined that these phases are concurrent but independent of each other; as such any agent which accelerates the process is a promoter of wound healing (Briggs & Flemming, 2007).

When wound occurs, it is accompanied (within a short time) by pain, reddening and oedema, which are the classical symptoms of inflammation. These symptoms are caused by the release of eicosanoids, prostaglandins, leukotrienes and reactive oxygen species (ROS). ROS is produced in high amounts at the site of wound as a defense mechanism against invading bacteria. At the same time, the process of wound healing may be hampered in the presence of free radicals (which can damage wound surrounding cells) or by microbial infection (Ilango & Chitra, 2010).

Wounds and particularly chronic wounds are major concerns for the patients and clinicians alike; chronic wounds affect a large number of patients and seriously reduce their quality of life. Current estimate of chronic wounds was reported to stand at 6 million people (Sasidharan et al., 2010).

2.1.1. Wound healing promoters research

Research on wound healing promoters was reported to be one of the developing areas in modern biomedical science (Sasidharan et al., 2010). Many traditional practitioners across the world particularly in countries like Malaysia, Saudi Arabia, India, Africa and China with ancient traditional practices have valuable information of many lesser-known hitherto unknown wild plants used for treating wounds and burns.

Kumar et al., 2007; Maurya & Maurya, 2012; Sasidharan et al., 2010 reported the use of several drugs from plants, minerals and animals origin in Indian systems of medicine like Ayurveda for their healing properties under the term '*Vranaropaka'* .

It has been documented that herbal medicine from certain plants and animal products were used for treatment of cuts, wounds and burns. Some of these plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models and human subjects, but potential from most of the plants remain unexplored(Maurya & Maurya, 2012).

Sasidharan et al., 2010 studied the wound healing activity of leaves of *Elaeis guineensis* (palm oil tree) by incorporating the methanolic extract in wound induce experimental rats. They reported that the extract showed significant activity against *Candida albicans* with a minimum inhibitory concentration (MIC) value of 6.25 mg /mL , results showed that the *E. guineensis* extract has potent wound healing capacity, as an evident from observed better wound closure, improved tissue regeneration at the wound site, and supporting histopathological parameters pertaining to wound healing .Similar observation was reported by (Agarwal et al., 2009) while observing the wound healing activity of plantain banana extracts in rats.

Ilango & Chitra, 2010 studied the wound healing and anti-oxidant activities of methanol extract of *Limonia Acidissima* Linn (Rutaceae) fruit pulp in rats. After treatment of the experimental groups they observed increased wound breaking strength, decreased epithelization period, increased wound contraction, increased granulation tissue weight and hydroxyproline concentration were observed in the various groups were compared with the control group. Also, increased activity of anti-oxidant enzymes and catalase levels, were also reported in extract-treated groups when compared to controls. Wound healing activity was statistically significant in animals treated with 400 mg/ kg of the extract. Based on their observations they concluded that methanol extract of *L. acidissima* possesses significant dose-dependent wound healing and anti-oxidant activities, which further supported the traditional claim of this plant as a wound healer.

Wound healing and antioxidant activities of *M. citrifolia* leaf extract in rats was studied by Rasal et al., 2008.They administered 20 mg/ kg aqueous extract of leaves of *M. citrifolia* on excision. They studied the incision resutured, wounds contraction, changes in blood malondialdehyde (MDA) levels and they also carried out histopathological studies. They reported significant increase in wound contraction rate, tensile strength, granuloma breaking strength, collagen content, dry granuloma weight and hydroxyproline content. When compared control group with the experimental group, they observed significant decrease in epithelialisation period and MDA levels in *Morinda citrifolia* leaf extract treated group. Therefore, based on their observations and statistical analysis they reported that probably the *M. citrifolia* aqueous leaves may enhances the wound healing and possesses antioxidant activity (Rasal et al., 2008).

As it has been mentioned above, wound infection is one of the most common diseases in developing countries because of poor hygienic conditions (Kumar et al.,

2006). They are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (Umachigi et al., 2007). Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue (Raina et al., 2008). Medical treatment of wound includes administration of drugs either locally (topical) or systemically (oral or parenteral) in an attempt to aid wound repair (Murti et al., 2011). The topical agents used include antibiotics and antiseptics (Gupta & Jain, 2010), desloughing agents (chemical debridement, e.g. hydrogen peroxide, eusol and collagenase ointment, wound healing promoters (e.g. Tretinoin, plants extract, honey, comfrey, benzoyl peroxide, chamomilia extract, dexpanthenol, tetrachlordecaxide solution, clostebol acetate and the experimental cytokines (Raina et al., 2008).

Yeo et al., 2011 reported that wound healing takes place in several processes which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and acquisition of wound. Acute wounds normally heal in a very orderly and efficient manner characterized by four distinct, but overlapping phases: Homeostasis, Inflammation, Proliferation and Remodeling (Braiman-Wiksman et al., 2007). The normal healing response set-in once the tissue is injured. The platelets come into contact with exposed collagen and other elements of the extracellular matrix as a result of the blood and its components spill into the site of injury. These results in Homeostasis where by chemical signals are released by the bacteria which attract the neutrophils enter the wound site and begin the critical

task of removing foreign materials, bacteria and damaged tissue (Borkow et al., 2008).The process of epithelization is stimulated by the presence of EGF (epidermal growth factor) and TGF-α (transforming growth factor alpha) that are produced by activated wound macrophages, platelets and keratinocytes. Once the epithelial bridge is complete, enzymes are released to dissolve the attachment at the base of the scab, resulting in its removal (Borkow et al., 2010).

2.1.2. Optimum Environment for Healing

Optimum environment is necessary for wound healing acceleration. Moist environment of the wound is required as it has been reported to enhance the natural autolysis processes and breaking down of necrotic tissue (Diegelmann & Evans, 2004). Although the wound surface should remain moist, excessive exudates should be removed from the wound but not allowing the wound to dry out, so as to maintain a moist environment (Diegelmann & Evans, 2004). Gaseous exchange should be allowed so that oxygen, water vapor and carbon dioxide can pass into and out of the wound. Oxygen was reported to be essential at all stages of healing. The oxygen concentrations affect the rate and density of capillary growth. However, in many occasions, the presence of exudates or debris on the wound may inhibit gaseous exchange (Lloyd et al., 2009). Lack of oxygen in the wound creates an area of low oxygen tensions thus stimulating the growth of capillary loops into the wound (Lloyd et al., 2009). It is also reported that maintaining constant temperature at 37°C promotes both macrophage and mitotic activity during granulation and epithelialization . The wound also should be prevented from either particulate or toxic contamination (Nigam et al., 2010).

In generally, it has been observed that clinically, one often encounters non-healing, under-healing or over healing. Therefore the aim of treating a wound is to

either shorten the time required for healing or to minimize the undesired consequences (Sato et al., 2010). As such Attention should be directed towards discovering an agent, which will accelerate wound healing either when it is progressing normally, or when it is suppressed by various agents like corticosteroids, anti-neoplastics, or non-steroidal anti-inflammatory agents (Raina et al., 2008).

2.1.3. Physiology of wound healing

Physiologically wound healing processes can be divided into three phases toward final repair. These are a) inflammatory phase, b) Fibroblastic/ inflammatory phase and c) remodeling phase (Barrientos et al., 2008).

(a) Inflammatory Phase

Inflammation is a normal process and among the prerequisite to healing. This response is a non-specific local reaction to tissue damage and or bacterial invasion aroused by the body's defense mechanisms and is an essential part of the healing process (Sato et al., 2010). Tissue damage triggers both the complement and cytokine systems. The complement system consists of plasma proteins, which are inactive precursors. When the precursor activated, there is a cascade effect that leads to the release of histamine from the mast cells and results in vasodilatation and increased capillary permeability. This effect is enhanced by kinin system that activates kinogen to kinin. Kinin will attract neutrophils to the wound, enhance phagocytosis and cause pain by stimulating the sensory nerve ending (Frey et al., 2006).

As the capillary dilate and become more permeable, fluid flows into the injured area and become inflammatory exudates, which contains plasma proteins, antibodies, erythrocytes, leucocytes and platelets. Platelets involved in clot formation and releasing

growth factor and fibronectin that enhances neutrophils motility, chemotaxis, adhesion to endothelial cells and necessary in both cell migration and phagocytosis (Majewska & Gendaszewska-Darmach, 2011). The presence of leucocytes at the wound removes bacteria and other foreign bodies by phagocytosis.

(b) Fibroblastic / proliferative Phase

Fibroblastic phase take place when the inflammatory phase completed to rebuild, resurface and impart strength to the wound. Usually occur between days 4-14 after injury. Epithelialization, angiogenesis, granulation, tissue formation, and collagen deposition are among the major steps of this phase (Diegelmann & Evans, 2004). The undamaged epithelial cells within the wound margin start to reproduce within hours after injury. Endothelial cells produce vascular endothelial growth factor (VEGF), FGF, and PDGF. Keratinocytes produce TGF-α, TGF-β, and KGF (Majewska & Gendaszewska-Darmach, 2011). The process of epithelialization is impaired by the presence of necrotic tissue, or slough, which may need to be actively removed by debridement to promote healing (Davies et al., 2005; Ramundo & Gray, 2008). Epithelialization can be facilitated by maintaining moist dressings, protecting the wound from minor repetitive trauma, avoidance of chemical irritants or infection, debridement and possibly topical oxygen therapy (Eaglstein, 2008). Through the process of contraction, the entire wound is pulled together and become shrink. Successful contraction results in a smaller wound to be repaired by scar formation, which gradually flattens to a thin white line (Mahmood et al., 2010).

The climax of wound healing occurs with collagen production. If this event does not occur, the wound will not heal (Barrientos et al., 2008). As collagen is laid down, the tensile strength of the wound increases (Barrientos et al., 2008). Fibroblast originates

from mesenchymal cells located in loose tissue and around blood vessels and fats. In response to chemotactic influence from the injury, these precursors of the fibroblast transform into cells with migratory ability. The fibroblasts synthesize collagen molecule, FGF, TGF, PDGF, insulin-like growth factor 1 (IGF-1), keratinocyte growth factor (KGF) and glycosaminoglycans (GAG), which fill in the space between and around collagen fibers. This GAG ground substance, combined with water, provides lubrication and acts as a space between moving collagen fibers (Barrientos et al., 2008). All these growth factors are needed to continue stimulating wound cellular proliferation, angiogenesis, production of extracellular matrix proteins and glycoproteins at the wound site.

(c) Remodeling phase

This is the final stage of wound healing process is remodeling phase which is also known as maturation phase. Clinically, this stage is the most important because the main feature of this phase is deposition of collagen in an organized network. For a successful wound healing not only closing the wound with sufficient tensile strength is important but also the ultimate goal normal functioning. Remodeling requires the scar to change to fit the tissue.The scar remodeling is responsible for the final aggregation, orientation and arrangement of collagen fibers. Unfortunately, the process of scar remodeling is not fully understood (Diegelmann & Evans, 2004).

Some researchers observed that remodeling of the scar tissue leads to a scar that is a vascular in nature, which therefore appears paler than the surrounding tissue. The scar tissue usually appears flattened compared to normal tissue and it does not contain any sebaceous gland and hair follicle (Diegelmann & Evans, 2004). However, in hypertropic scar there is excess formation of scar tissue which cause the persistent

raised of the scar sometimes it may extend beyond the original wound into healthy tissue resulting in a keloid scar (Diegelmann & Evans, 2004).

2.1.4. Antioxidant in wound healing

An antioxidant is a molecule that is capable of slowing and preventing the oxidation of a molecule. They are substances when present in low concentrations compared with those of the oxidizable substrate significantly delay or inhibit oxidation of those substrates. Antioxidants minimize the oxidation of lipid component in cell membranes or inhibit the volatile organic compounds and the conjugated dine hydroperoxides which are known to be carcinogenic (Amiri, 2009; Tepe et al., 2007).

Several studies demonstrated the beneficial effects of antioxidants in accelerating wound healing (Mahmood et al., 2010). Oxidative stress is thought to contribute to development of a wide range of diseases including Alzheimer, diabetes and neurodegeneration (Lee et al., 2007). When there is an imbalance between antioxidants and free radicals, oxidative stress will prevail. The free radicals that damage cellular macromolecules, producing oxidative stress, are scavenged in the human body by a range of antioxidant enzymes and small molecule antioxidants (Lee et al., 2007). Hence, Antioxidant activity is popularly used to indicate the ability of an antioxidant to scavenge some radicals. Free radical colorimeter relies on the reaction of an antioxidant with the stable free radical 2, 2-diphenyl-l-picrylhydrazyl (DPPH) dissolved in methanol(Lee et al., 2007). (KGF) is a relatively stable paramagnetic free radical that accepts electrons or H^+ radicals to become a stable diamagnetic molecule. The reduction of DPPH by an antioxidant results in the formation of a purple-blue colored solution which gives the measure of the activity by spectrophotometer at 541 nm (Lee et al., 2007).

2.2. Stomach

2.2.1. Rat Stomach

The rat stomach comprises of two regions: non-glandular (forestomach) and glandular part. The non-glandular stomach is lined by cornified squamous epithelium. The glandular stomach consists of corpus, antrum and pyrolus, is lined by a simple columnar epithelium. The non-glandular part is distinguished from the glandular part of the stomach by the limiting ridge, which prevents a reflux of gastric juice from the glandular part into the non-glandular part when the stomach is empty. The anatomy and physiology of the rat stomach is not much different from the human stomach. Many researches applied the rat stomach as an investigation tool to discover the gastric function, so the results from rat models provide the basic information for human (Kararli, 2006).

2.2.2. Peptic Ulcer and Gastric Ulcer Disease

There are various kinds of ulcers, and their names are named after the locations in the digestive tract. Duodenal ulcer or peptic ulcer can be found in the small intestines, while gastric ulcer is located inside the stomach. A gastric ulcer is considered as a necrotic lesion penetrating through the entire mucosal thickness of the stomach. The peptic and duodenal ulcers are usually benign; however gastric ulcers are more often to develop malignancy. In this study we will focus on the gastric ulcer induced by Hcl/ethanol**.**

2.2.3. Gastric damage

Gastric layers area interrupt by various exogenous and internal factors cause stretch on mucosa surface which lead ulcer in gastrointestinal surface. Once, the mucosal damage

occur usually accompany with secretion of acidity on surface and pepsin production enhance damage. This occurs between duodenum and gastric and rare appears upper part of gastric system below esophagus and lower jejunum(Ramakrishnan & Salinas, 2007b). Environmental and life style effects and raise the prevalence of ulcer within the population, occupation nature and geographical location. Peptic ulcer is amongst the worst and aggressive disease in the world(Falcão et al., 2008)

Stomach ulcer incidence is obvious statistically increasing with years, this has shown effect on quality of life in patients with disease, on basic health care provided and economic system in the country as general influence to all sectors necessary for better life(Yuan et al., 2006). Moreover, ulcer perforation it has been considered to cause mortality and morbidity (Duggan and Duggan, 2006).Gastric ulcer categorized as the predominant disease in digestive system in human (Falcão et al., 2008).

Stomach sore, losing of weight, cardiac pain, nausea, vomiting of hematemesis, foul smelling feaces (melena) are associate symptoms of ulcer in digestion system.(Ramakrishnan & Salinas, 2007a). Non steroid anti inflammatory drugs are the first line due to gastric ulcer(Bytzer & Teglbjærg, 2001). Avoidance of certain ulcerogenic agents and take completely rest, antiacid intake, PPI, stop smoking this is most path recommended for ulcer healing as prior surgery interference

2.2.4. Mechanical gastric ulcer

Recently, cause of imbalance between exogenous and aggressive factors trusted to be food and life stress as main reasons to develop mucosa stretch which lead to gastric ulcer. Hospitalization, rest in bed and special diet prescribed as part of good treatment to be given to the patients. Furthermore, HCL blamed to be ulcer necrotizing agent to cause wound ulcer in gastric. Proton pump inhibition drug become as the main to

treatment to block acid production. Recurrence prevalence is high of gastric ulcer after treatment due to incomplete healing entirely. Defensive and aggressive mechanisms are causative of severe ulcer and perforation in mucosal surface. The defensive factors are blood flow rate, prostaglandin and production of mucous, where the aggressive factors such as NSAIDs, HCL, *H.pylori* and secretion of pepsin (Tarnawski, 2005)

2. 2 .5. Cytogastroprotective agents

Stomach is a digestive system where all intake foods digest to chyme than move to duodenal tube for absorption and follow to be extracted as waste. It has been interested organ for many researchers since years to remark the entire mechanisms. Past decades studies contribute in preventing mucosa lining surface from necrotizing agents, significant cytoprotection substances promised to protect mucosal surface as anti ulcer mechanisms such PGE2 ,secretion of mucus act as barrier and internal mechanisms secret bicarbonate(Wallace, 2008).

Wallace and Granger explained mucosal defense as variety factors and secretion molecules that make protection on surface mucosa from pH, osmolarity and environmental cells toxicity (Wallace & Granger, 1996). Usually, mucosal damage exist in nature periodically, this doesn't interrupt the regular function of protection properties of mucosal epithelial and with no clinical remark as symptoms. This is because the structure of mucosal layers consist several of the primary and secondary which make more impotent exposure to external substances cause some breaching on surface, this following with tissue repair mechanisms of epithelial surface tissue scratch(Wallace & Granger, 1996). Moreover, Endogenous molecules can altered cytoprotection defensive by suppressing or blocking such as prostaglandin enzymes (PGE2) (Wallace, 2008).

Researchers such as vane & botting were able to define cytoprotective of gastrointestinal system mechanisms of prostaglandin.They hypothesis the relation between the administrations of NSAIDs with secretion level of prostaglandin. In addition, explained in detail the drugs mechanisms to suppress defensive substances and damage of mucosal tissue(Vane & Botting, 1997).

Studies have been investigated the main prostaglandin, $PGE_{2,}$ $PGI_{2,}$ with minor quantity of $PGF_{2\alpha}$ and PGD_2 (Peskar et al, 1977; Peskar et al., 1980). PGE2 is part of mucosal defensive is capable to inhibit acidity secretion on mucosal epithelial surface. prostaglandin play a vital role in gastric prevention(Bianchi Porro et al., 2000). Postulated that IP and EP3 receptors are responsible to this action(Kato et al., 2005; Mutoh et al., 2002). Prostaglandins reported elsewhere to enhance production and activate molecules to secrete defensive mechanisms and is suppress inflammatory mediators regulation that involving in gastrointestinal damage(Martin & Walace, 2006). This suppress histamine, TNF-α and PAF which involve in mucosa damage (Hogaboam et al., 1993). Vascular endothelial growth factor (VEGF) released by PGE2 (Prostaglandins) as enhance recovery to ulceration.(Miura et al., 2004)

As described above, several defensive mechanisms available at the different level of mucosal damage. Gastric epithelial layer mainly act as the first line defense against aggressive factors manifest as mucus barrier to bounce the penetration.(Wallace, 2008). Carbohydrates and amino acids are component substances released by the mucous cells on superficial mucosa epithelial cells.(Singh et al., 2005). This stabilize the mucus on the gastric mucosa and generate defensive mechanisms(Ho et al., 2004).Is essential barrier(mucus substance) on gastri mucosa layer to stabilize pH and against pepsin enzyme(Allen & Flemström, 2005). Furthermore, mucus substances on epithelial tissue

and luminal tissue are creating moisture environment to minimize attachment of pathogens or intake materials.(Moncada et al., 2003).

2.2.6. Aggressive Factors

Prolong the administration of non steroid anti inflammatory has adverse reactive against inhibition of other defensive molecules mechanisms this contribute in ulcer formation.

High prevalence in prescribed medicines is NSAIDs and addicted to these drugs are range between 15~30% involving with gastric ulcer and massive breach bleeding in mucosal tissue(Mizushima & Yoshimori, 2007). Non steroid anti inflammatory drugs mechanisms on gastrointestinal damage permeability membrane and suppressing of cyclogxygenase enzyme activity figure 2.1, direct contributing to gastro epithelial prevention by rising pH and turn up mucous/bicarbonate this turning on blood flow and epithelial tissue on proliferation to repair any damage caused(Huang et al., 2002).

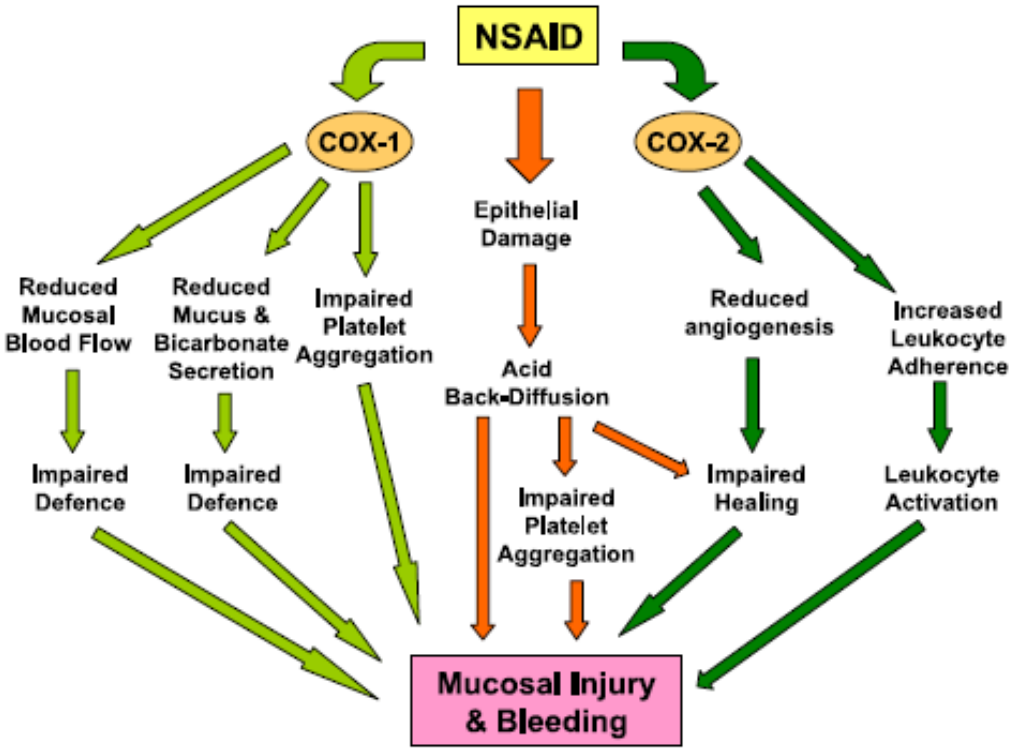

Fig 2.1: Fig 2.1: Non steroid anti-inflammatory drugs pathogenesis (Wallace, 2008)

Bacterium *H. pylori* shaped rod and cause of gastrointestinal ulcer, found in human gastric tissue (Nanjundaiah & Annaiah, 2009). *Helicobacter pylori* in addition to NSAIDs are main cause of gastrointestinal ulcer in human being. Both of them have something like synergy to each other to cause ulceration and hemorrhage lesion(Huang et al., 2002). *H.pylori* is gastriculcerogenic agents by attached on mucosal receptors, recurrent of disturbing mucosal surface by adherence, exist the pathogenesis proteins and act as cytotoxic related to gene islands in the bacterium chromosomes this contribute indirect in ulcerogenic factor (Nilsson et al., 2003). Moreover, infected patients with this bacterium are activating gastric intestinal enzymes and down regulate production of mucus/bicarbonate releasing. All factors are reason of ulcer formation. Eradication of this pathogen will assist to reduce recurrent of any future possibly to develop ulcer up 67% (Hopkins et al., 1996).

Free radicals have been reported as the main causative factor in the occurrence of pathogenesis in human diseases, such as gastrointestinal dysfunctions, atherosclerosis, tumor promotion and carcinogenesis, hemorrhagic shock, arthritis, Alzheimer and Parkinson's disease, ischemia and reperfusion injury (Bagchi et al., 2000).The oxidation agents are categorized as biochemical substances that have incomplete electron pairs in their exterior electronic shell site (Martini et al., 2000). These radicals acquired by attacking proteins, DNA and fatty bodies on cell membrane to release electrons to complete a missing pair (Halliwell & Gutteridge, 1989).

Studies confirmed that ethanolic agent participating in free radicals proliferation. reported that EtOH contribute to free radical production. Navasumrit deduced vitamin C and E prevented completely the hepatic DNA strand breaks induced by EtOH due the

efficacy of these antioxidants in inhibiting the production of hydroxy free radicals in induction of acute EtOH state (Navasumrit et al., 2000).

Gastric ulcer damage induced with induction of ethanol is regarded as a typical ulcerogenic design commonly used for the evaluation of antiulcer activity of compounds and natural products (Almeida, 2011). Researchers reported that EtOH is able to generate numerous cellular responses that are enough to induce gastric ulcer and suppress the antioxidative defensive mechanism of the gastric mucosal cells (Gonthier et al., 2004).

Necrotizing agents that induce ulcer, such as ethanol, has been assumed in several mechanisms: (1) Insufficient gastric mucosal blood vessels circulation that results in necrosis and mucosal bleeding (Srikanta et al., 2011). (2) Rapid penetration of EtOH through gastric mucosa, resulting in peeling of gastric mucosa and the release of vasodilation molecules exerted by neutrophils infiltration cells and reduction of blood flow in gastric mucosa rising development of ulcer (Bilici et al., 2002). (3) Ethanol produces reactive oxygen stress (ROS) through production of $O^{-\cdot}$, H_2O_2 free radicals causing cell membrane damage (Alqasoumi et al., 2009). In addition, Xanthine oxidase and MDA increases and glutathione content in gastric mucosal cells decrease (Marotta et al., 2000).

2.2.7. The Epithelium

Although there are several “layers” of mucosal defense, experimentally reducing the effectiveness of the mucus-bicarbonate layer on the epithelial surface does not usually result in epithelial damage (Wallace, 2008). The underlying ability of gastric epithelial cells might play role to remain intact and functional when continuously

exposed to high concentrations of acid. Sanders and colleagues (1985) reported that the apical membrane of cultured chief cells was highly resistant to damage by acid and these cells can tolerant to a solution of pH 2 for more than 4 hour without cause any damage to the cells. Gastric-surface mucus cells have a mechanism to resist the back-diffusion of acid into the mucosa that involves enhancement of the functional barrier (Takezono et al., 2004).

2.2.8. Mucosal Blood Flow

Mucosal blood flow is rapidly triggered by sensory afferent neurons when irritants such as acid enter the sub-epithelial compartment, allowing the buffering of acid and the rapid removal of toxic substances, thus limiting their penetration into deeper layers of the mucosa (Wallace & Devchand, 2009). When the sensory afferents are stimulated, it will cause a rapid increase in mucosal blood flow. (Whittle et al., 2012) reported that excision of the sensory afferent neurons cause the abolishment of the “reactive hyperemic” response to topical irritants and also increases the chances of the gastric mucosa to injury. A constant delivery of plasma from the sub-epithelial blood vessels plays important role in the maintenance of a repair-conducive microenvironment. Interruption of blood flow can result in a rapid decrease in the pH at the site of injury which leads to disruption of the repair process and progression of damage to deeper layers of the mucosa (Wallace & Ferraz, 2010).

2.2.9. Gastric ulcer Healing

Healing of a gastric ulcer means a restoration of epithelium, endothelium, connective tissue, and smooth muscle that have been damaged during ulceration. Ulcer healing is a complex repair process that involves inflammation, cell proliferation

(particularly at the ulcer margin), formation of granulation tissue at the base of the ulcer, and angiogenesis (new blood vessel growth). Normally, intestinal epithelial repair after wounding involves different processes which can be believed as partially independent (Fig. 2.2). The rapid response to damage that involves migration of surrounding epithelial cells to cover the denuded area(Narkar et al., 2008) and this process is called re-epithelialization. The crucial processing due its capabilities to prevent granulation tissue from infection, chemical and mechanical damage.For small wounds, short restitution and proliferation phases may be sufficient to restore the monolayer. If the wound is large, immunologic responses and deposition of protective granulation tissue may be necessary to restore epithelial continuity.

The epithelization and migration leads activation and regulation of transcription and growth factors(Tarnawski, 2005, 2010)

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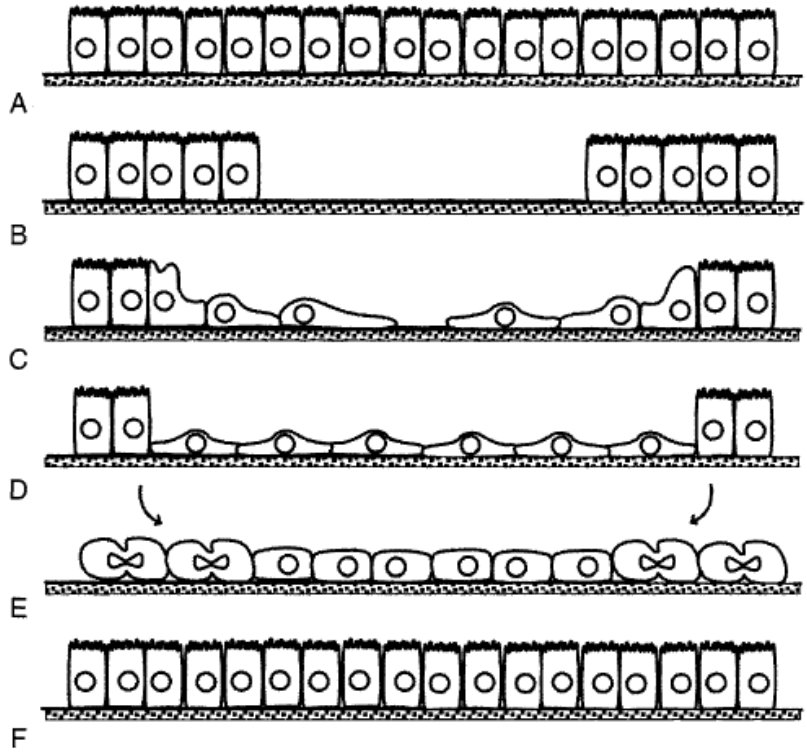

Fig 2.2: The basic processes of intestinal epithelial wound healing. When an undamaged mucosal surface (A) is subjected to an insult that strips away epithelial cells (B), the first response is loss of polarization of cells near the wound margin and conversion to a migratory phenotype (C). Over a process of minutes to hours, depending on the extent of the wound, cells flatten and move to cover the denuded area, re-establishing the protective barrier (D). Leader and follower cells often move as a unified sheet, maintaining rudimentary attachments during the restitution process. Cell proliferation restores the epithelial population (E), allowing cells to reform normal junctional complexes and retrieve a polarized columnar phenotype (F) (Frey et al., 2006).

Granulation tissue develops at the ulcer base in two or three days after ulceration (Tsukimi et al., 2008; Tsukimi & Okabe, 2008).Connective tissue regeneration found in granulation tissue, form micro-vessels through the process of angiogenesis. Granulation tissue plays crucial role in healing process of ulcer because it provide extracellular

matrix proliferation linked with lamina propria recovery. The micro-vessels proliferated to facilitate microvascular restoration (Tsukimi et al., 2008; Tsukimi & Okabe, 2008).

Angiogenesis is the process to form a new microvascular network, which consider is an essential for the healing of chronic gastroduodenal ulcers. Angiogenesis happens through a serial steps, which include: (a) degradation of capillary basement membranes by matrix metalloproteinases, (b) endothelial cell migration into the perivascular space and proliferation, (c) formation of microvascular tubes followed by anastomoses, (d) establishment of lumina and basement membranes, and ultimately (e) formation of the capillary network (Lazaraki et al., 2008). The formation of granulation tissue and generation of new microvessels through angiogenesis is induced by bFGF, VEGF, PDGF, angiopoietins, and other growth factors and cytokines, including IL-1 and tumor necrosis factor-alpha (TNF-α) (Tarnawski, 2005).

Extracellular matrix (ECM) is secreted by a wide range of cell types including fibroblasts, epithelial, smooth muscle and endothelial cells and assembles into a network in the spaces surrounding cells which withdraws water and minerals and binds growth factors (S Tarnawski & Ahluwalia, 2012; Tarnawski). ECM comprises of fibrous structural proteins such as the collagens and elastins, adhesive glycoproteins including fibronectin and laminin and an amorphous gel composed of proteoglycans and hyaluronan. All the components above can form an interstitial matrix and the basement membrane (Tarnawski, 2005).

The replacement of granulation tissue with a connective tissue scar changes the composition of the ECM. The growth factors not only stimulate production of collagen and other connective tissue components but also modulate the synthesis and activate of metalloproteinases, enzymes which degrade these ECM components. The result of this

process is remodelling of the connective tissue that is an important feature of ulcer healing. Degradation of the collagen and other ECM proteins is accomplished by the matrix metalloproteinases, enzymes dependent on zinc ions for their activity. Some collagenases and their inhibitors are essential in the remodelling of connective tissue necessary for the tissue defect repair and scar formation (S Tarnawski & Ahluwalia, 2012; Tarnawski, 2005).

2.2.10. Peptic Ulcer Treatment

Many drugs have been approved by FDA for treating peptic ulcer. These drugs are selectively inducers to specific mechanisms that are related to peptic ulcer therapy such as mucosal defenses enhancement or gastric acid neutralization. In 1988, omeprazole has been introduced as a proton-pump inhibitor (PPI) and found to be the drug of choice for treating peptic ulcer (Lin, 2010).

Omeprazole (Fig. 2.3) is a proton pump inhibitor that inhibits gastric acid secretion by suppressing ATP enzymes system and increase mucus production. It is used in the treatment of gastric disorders such asgastroesophageal reflux disease, dyspepsia and peptic ulcer, including that caused by the infection with *Helicobacter pylori*.

. In addition, patients are used to combine omeprezole with clarithromycin, metronidazole and amoxicillin to treat *H. pylori*-induced duodenal ulcers (Majumdar et al., 2011).

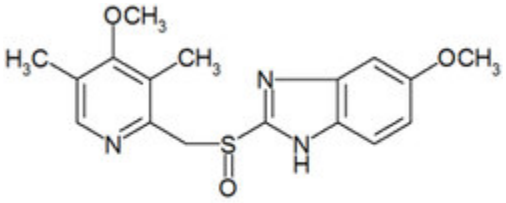

Fig. 2.3: Chemical structure of omeprazole 5-methoxy-2-[(4-methoxy-3,5-dimethyl-pyridin-2- yl)methylsulfinyl]-3H-benzoimidazole (Lindberg et al., 2006)

It has been used to treat various complication as gastroeasophagial disease,up regulate secretion,and gastric haemmorrhage(Makola et al, 2007), Omeprazole should be taken not for long term associated with hyperplasia,atrophic gastritis,vitamin complex malabsorption and leading to cancer development (Klinkenberg-Knol et al., 1994; Lin, 2010).

2.2.10.1. Characteristics of Omeprazole

Omeprazole has some unique features that are important for its mechanism of action. One of the characteristics of omeprazole is, it is lipophilic and it can easily penetrate cell membranes. Besides that, omeprazole is a weak base which means that it concentrates in acid compartments. Thirdly, omeprazole is unstable in acidic solution. The half-life of omeprazole at pH 1 is approximate two minutes, and when it exposes to neutral solution, that is pH 7.4, it can last for about 20 hours. Therefore, omeprazole is a

drug that accumulates within the acid space of the target cell, where it is transformed to the active inhibitor (Sohi et al., 2010).

2.2.10.2. Limitation of Omeprazole

Toxicological studies of very high doses of omeprazole in rats could lead to the formation of endocrine tumours (carcinoids) in the stomach. The carcinoids arose from entero-chromaffine-like (ECL) cells, a type of endocrine cell in the gastric mucosa that synthesize and produce histamine, which process stimulate by gastric hormone gastrin (Larsson *et al*., 1986). Nevertheless, longer term stimulation by gastrin has a massive effect on ECL cells. As amount of gastrin increases and decreasing amount of acid secretion, this observed effect will result in elimination of gastric acid secretion, leading in massive hypergastrinemia (Ally et al., 2009).

2.3. Research Animals

For a very long time, scientific experiments have been performed on animal to provide an insight into health and diseases, and in the quest for new treatments and cures. By the nineteenth century, there were enough knowledge to show the striking similarities between animal and human in nutrition and physiology. This led to the idea that animals could be used as disease models for humans. The response of an animal to a chemical, potential medicine was thought to the same response in human (de Jong & Maina, 2010). Laboratory animals are used in four main areas, which are in biomedical research, in producing of natural products in medical treatment, testing for safety and toxicity and in education. Animal experiments have contributed to major medical advances like discoveries of antibodies, vitamins, and tumors. They also contributed in development of antibiotic anesthetics and medicine for treating diabetes, cancers and asthma and production of vaccines for polio, rubella and whooping cough. The apparent

adaptability of mice and rat made them an ideal choice for breeding and maintenance in captivity. This characteristic resulted in their successful development as species of choice in many laboratories. They were omnivorous, robust, prolific, and small and require less time of breeding. Therefore, they were cheap and easy to breed and maintain. These small rodents took their place in the forefront of laboratory animal hold to the present day (de Jong & Maina, 2010).

2.4. Medicinal Plants

For centuries, Plants are still good source of food to human. Also, many other cosmetics/medicines come from natural products(Harborne & Baxter, 2001). WHO (world health organization,geneva) is strongly encourage natural products research to achieve safe and cost cutter medicines to infection and non-infection diseases(Principe, 1985).

Furthermore, Researchers are so curious to learn more on phytochemical and pharmaceutical interest of isolated compounds from the natural products. In addition, addition to natural medicine about 25% of active compounds were studied in details their pharmaceutical activity(Falcão et al., 2008; Pezzuto, 1997).

Amongst top twenty drugs in 1996 were sold and about 50% were represent natural products medicines(Balandrin et al., 1985). Medicinal source of important therapy comes from natural products which treat various diseases.(Falcão et al., 2008; Pezzuto, 1997)

2.5. Selected Malaysian Medicinal Plants

2.5.1 *Jasminum Sambac* Linn. (Arabian Jasmine)

Jasminum sambac. **Linn (Oleaceae)** is commonly known as Jasmine (Fig.2.3). Traditionally, *J. sambac* is used as the treatment of various illnesses such as rheumatism, paralysis, gallstones and diabetes mellitus. Flowers of *J. sambac* are used to reduce fever, swollen eyes and bee stings. Leaf part is usually used to reduce the shortness of breath and as treatment of acne. The root part is used to treat headache, insomnia and is believe can accelerate fracture healing. Essential oil of *J. sambac* is used as fragrance for skin care products, tones the skin as well as reducing skin inflammation (Abdoul-Latif et al., 2010). *J. sambac* flowers and leaves are largely used in folk medicine to prevent and treat breast cancer. Flowers of *J. sambac* are useful to women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding (Kalaiselvi & Kalaivani, 2011). Previous studies done on *J. sambac* reveals that the plant contains antifungal (P. Singh et al., 2011), anti-cancer (Talib & Mahasneh, 2010). Essential oil and methanol extract from *Jasminum sambac* have *in vitro* antimicrobial and antioxidant activities which could support the use of the plant by traditional healers to treat various infective diseases (Abdoul-Latif et al., 2010). Phytochemical studies shown that the roots contains dotriacontanoic acid, dotriacontanol, oleanolic acid, daucosterol and hesperidin (Rahman et al., 2011). Ethyl acetate and water extract of leaves of *Jasminum sambac* showed reduction in plasma glucose level, lipid profile and serum urea in diabetic rats (Zhang et al., 1995). However, there is no data reported on anti-ulcer activities within the plant.

Fig. 2.4: *Jasminum sambac*. Linn (Oleaceae) (http://plants.usda.gov)

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Asteridae*
Order	*Scrophulariales*
Family	*Oleaceae* – Olive family
Genus	*Jasminum* L. – jasmine
Species	*Jasminum sambac* (L.) Aiton – Arabian jasmine

2.5.2. *Hemigraphis colorata* (Blume) H.G. Hallier (Acanthaceae)

H.colorota is a tropical planted front of the houses for decoration purpose.In fact; phytochemical of this plant intenstively studied done using different solvents.Leaves/crude extracts show the presence of various compounds such as alkaloids, phenolic compounds, flavanoids, carbohydrates, carboxylic acid, saponin, benzene, and tannis. It suggested to use it as antimicrobial,antioxidants,anticancerous antidibetic(Anitha et al., 2012).

Since time immemorial, man has used various parts of plants in the treatment and prevention of many ailments (Chah et al., 2006). Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixture. Today a substantial number of drugs are developed from plants (Fabricant & Farnsworth, 2001) which are active against a number of diseases. The majority of these involve the isolation of the active ingredient found in a particular medicinal plant and its subsequent modification. Many higher plants contain novel metabolites with antimicrobial and antiviral properties (El-Shemy et al., 2007).

In the developed countries, 25 percent of the medical drugs are based on plants and their derivatives (P. P. Principe, 1996) and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries. Because of that, in Asian countries, leaves were used to treat cuts, rheumatisms and ulcers(Kalita et al., 2011).

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Fig. 2.5: *Hemigraphis colorata* (Blume) htttp://plants.usda.gov/java/

Kingdom	Plantae
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Scrophulariales
Family	Acanthaceae

2.6. Free Radicals

A free radical is chemical species capable of independent existence that has one or more unpaired electrons and is often described by the insertion of the radical dot to indicate that one or more unpaired electrons is present. Usually, radicals are less stable than nonradicals, they can react either with another radical or with another molecule by various interactions to achieve in stable state (Close & McArdle, 2007).

2.7. Reactive Oxygen Species (ROS)

Aerobes require oxygen (O_2) to perform cell function as it plays important role in a series of biochemical reactions such as electron transport chains and other enzyme systems. A series of electron transfer reactions produces several molecules of adenosine triphosphate (ATP), which provides the energy required for a multitude of cellular reactions and functions, for each electron that passes through the respiratory chain. Molecular oxygen can pair with four electrons, one at a time, and the corresponding number of protons to yield two molecules of water. Different oxygen radicals are formed as intermediate products during this process, that is superoxide (O_2^-); peroxide ($O_2^{=)}$, which normally exists in cells as hydrogen peroxide (H_2O_2); and the hydroxyl radical (•OH). Superoxide, peroxide, and the hydroxyl radical are considered the primary reactive oxygen species (ROS). Reactive oxygen species (ROS) is often referred to not only the radicals OH·, RO_2·, NO· and O_2·$^-$ but also the non-radicals HOCl, $ONOO^-$, O_3, and H_2O_2 (Close & McArdle, 2007).

An imbalance between production of ROS and activity of the antioxidant defenses is named as oxidative stress. Oxidative stress, superoxide production and an imbalance in antioxidant enzymes could contribute to the initiation of cancer (Reuter et al., 2010),

cause structural alterations in DNA (Reuter et al., 2010), and affect cytoplasmic and nuclear signal transduction pathways (Federico et al., 2007).

2.8. Antioxidants

Antioxidants are compounds which neutralize the free radicals generated by cellular activity.These compounds protect the organs from damage and scavenging free radicals results from tissue membrane activity. Free radicals are the reason of many diseases such as neurological diseases,ageing, gastrointestinal ulcer, genes defects,anemia,heart and all under inflammation complications disease (Bagchi et al., 2000).

Moreover, antioxidants categorized into hydrophilic(water like) and hydrophobic(lipid soluble) antioxidants whether soluble in water or in lipid.Soluble-water antioxidants scavenging radicals in cells and blood.On the other hands,antioxidants soluble in lipid react with lipid peroxidation from cell membranes(Sies, 1997). These compounds can be acquired by nature in our body or from food(Vertuani et al., 2004).

In nature, antioxidants divided into two groups as acquired inside our body synthesis GPx, SOD, and catalase are soluble in water and lower molecular weight. Scientists discovered that the antioxidants can be isolated from various plants such, vegetables, rose, plant leaves, weeds and seeds(Blomhoff, 2005). Green plants content more flavonoids which has been reported elsewhere has strongest antioxidants activity (Rai et al., 2006). Isolated compounds from plants such as flavones and flavanoids are contributed as secondary metabolitics as anti-radical properties in add to anti-oxidation. (Makari et al., 2008; Zargar et al., 2011).

Living organisms are inbuild machine to support against free redicals as reactive oxygen stress such as systematic enzymes or non systematic enzymes defensive.Moreover, defensive factors (endogenous) and aggressive factors (exogenous) can interrupt the protection activity which lead to imbalance of redox to unstable status.Hence, Disturbance of redox equilibirium in healthy person occurs on set of protecting oxidative reaction by antioxidants during the scavenging of oxygen stress species(Olalye & Rocha, 2007).Researchers are focusing in this era of antioxidants and pay attention on strong antioxidants compounds like flavonoids,all phenolic compounds a part from synthetic active compounds(Wegrzyn et al., 2008).The antioxidative effects of natural phenolic compounds are in pure forms or in their extracts from different model systems of oxidation (Kaur & Kapoor, 2008). Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of carcinogenicity, cardiovascular and neurodegenerative changes associated with aging (Talalay & Dinkova-Kostova, 2004). Several survey in public health studies show that consumption of natural products from plant originate can prevent us from many encounter diseases against ROS by scavenge the redicals stress.(Oboh & Ogunruku, 2010; Oboh et al., 2007). In nature exist of essential antioxidants with wide range of medical and pharmaceutical important have brought attention to act as anti-cancer, curing to all type of inflammatory diseases and ageinging preventive properties(Cho et al., 2006; Yoo et al., 2008). Researchers are still investigating medicinal plants and other natural products such as different crops inorder to identify the potential pharmaceutical antioxidants(Chen et al., 2009). Phenolic compounds exist from natural products show prove might reduce the ROS by other mechanical pathway.Hence,Isolated flavonoids from the natural products have exert indirect pathway mechanism effect antioxidants(Groot & Rauen, 2009).

2.9. Lipid Peroxidation

The free-radical oxidation of polyunsaturated fatty acids in biological systems is known as lipid peroxidation. Lipid peroxidation is an inevitable process *in vivo.* Cardiovascular diseases, such as preeclampsia and atherosclerosis are induced by this process and the end-products of this process such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) can cause damage to proteins and to DNA. Lipid peroxidation causes damage on biological membrane functioning, e.g., decreases fluidity, inactivates membrane-bound enzymes and receptors, and may alter nonspecific calcium ion permeability (Sachdanandam, 2006).

Lipid peroxidation induced by oxygen free radicals is believed to play important part in destruction and impairment to cell membranes, because this process degrades polyunsaturated fatty acids (PUFA) of the cellular membranes with consequent disruption of membrane integrity (Lobo et al., 2010).

Previous studies have reported that lipid peroxidation plays a significant role in the pathogenesis of gastric mucosal lesions induced by water immersion restraint stress, burn shock, and ischemia-reperfusion (Jia et al., 2007; Jiang et al., 2005; Naito & Yoshikawa, 2006).

2.10. Heat Shock Protein

The heat shock response was first reported by (Ritossa, 1962), who observed in *Drosophila melanogaster* chromosomes after heat treatment of the flies. The heat shock response then increases expression of genes encoding a special class of proteins which called the heat shock proteins (HSPs) or stress proteins.

Heat shock proteins play important roles in normal conditions and pathological situations involving both systemic and cellular stress. Previous studies have showed that most HSPs have strong cytoprotective effects, are involved in many regulatory pathways, and act as molecular chaperones for preserving important cellular proteins (Arya et al., 2007; Barrier et al., 2009). Thus, HSPs are essential for the maintenance of gastric mucosal cell integrity during both normal cell growth and engaged in several pathophysiological conditions (Laine et al., 2008).

Heat shock proteins are also implicated in gastric cytoprotection. HSPs are a group of highly conserved proteins that are induced in response to heat and other stresses (Schmitt et al., 2007; Van Eden et al., 2005). They are classified into subfamilies according to their molecular weight, such as small HSPs (16–30 kDa), HSP40, the predominantly mitochondrial HSP, HSP60, HSP70, HSP90 and the large HSP110. 70 kDa heat shock proteins assist in both the assembly and transport of newly synthesized proteins within the cells and also the removal of denatured proteins. Therefore, HSP70 plays a role in preventing damage and in cellular repair processes after injury. Previous studies also found that induction of HSP70 in response to heat, ethanol, oxidative stress, or water immersion–restraint stress in the gastric mucosal cell (Innes, 2012; Otaka et al., 2007). However, only few studies reported the localization of HSP70 in the injured stomach.

2.10.1. Heat Shock Proteins and Stomach

Stomach is one of the organs in our body that often exposed to outside irritants such as hot food, alcohol, and oxidants generated from ingested food and *H. pylori*-associated infection. Alcohol is one of major factors that cause gastric mucosal injuries including gastritis, ulcer, which may lead to gastric ulcer(Partida et al., 2009).Some studies showed that HSPs contribute to adaptive cytoprotection in the gastrointestinal mucosa. Among the HSPs, HSP70 is believed the one that play important role in the defense mechanism of gastrointestinal mucosa. It has been reported that HSPs are induced either in *in vitro* or *in vivo*. According to, HSPs are induced by heat shock treatment resist 7.5% ethanol in cultured guinea pig gastric mucosal cells. In an *in vivo* study, it was reported that overexpression of HSPs were induced by water-immersion restraint (WIR) stress in animals pretreated with hyperthermia, as compared with stress alone (Young Oh et al., 2005).(Choi et al., 2009a) revealed that oral administration of geranylgeranylacetone (GGA), one of the antiulcer drugs, produced HSP60, HSP70, HSC70, and HSP90 in the gastric mucosa of normal rats.(Park et al., 2008) also demonstrated that HSP70 inductions protected rats against ethanol-induced gastric mucosal damages.These results suggest that HSPs, especially HSP70, induce resistance of gastric mucosa against stress-induced mucosal damage. Thus, HSPs play a protective role in gastric mucosa under stressful conditions.

2.10.2. Heat Shock Proteins and Protection against Oxidative Stress

Heat shock proteins play important role in other cellular processes that occur during and after exposure to oxidative stress as well. Oxidative stress happens as a result of the imbalance between the productions of reactive oxygen species (ROS) and the detoxifying process of reactive intermediates. As a consequence, this imbalance generates excessive of ROS, which leads to oxidation and aggregation of vital proteins and DNA, causing failure of normal cell function (Kregel & Zhang, 2007).

Under this stress conditions, some HSPs, especially HSP70 family and its co-chaperones are synthesized at high level. Members of the HSP70 protein family include: HSC70 (a constitutive HSP70), present within the cytoplasm and nucleus; grp75, mitochondrial HSP70; grp78 (Bip), a resident of the endoplasmic reticulum (González & Manso, 2004).

The HSP70 protein family functions as molecular chaperones in refolding of denatured polypeptide (Mayer & Bukau, 2005). Molecular chaperone is defined as protein that binds to newly synthesized protein and refolds of denatured proteins (Young et al., 2004).

Members of the HSP70 family and its co-chaperones select and direct abnormal proteins to the proteasome or lysosomes for degradation(Mayer & Bukau, 2005). Previous studies revealed that overproduction of HSP70 was shown to reduce stress-induced denaturation and aggregation of certain proteins(Hwang et al., 2005; Kudryavtsev et al., 2012), which are another evidence to determine that HSP70 plays an important role in protection against stresses (Kalmar & Greensmith, 2009; Wang et al., 2004). Heat shock proteins may also show its protective effects against oxygen radical-

induced cellular damage such as membranes (lipid peroxidation), proteins, DNA, and mitochondria.(Trougakos & Gonos, 2006) reported the protective effects of HSP70 against lipid peroxidation and DNA damage. Therefore, over expression of HSPs may protect multiple cellular compartments and prevent protein damage from oxidative stress.

2.11. Bax protein

The balance between apoptosis and cellular proliferation is a main factor in gastric injury and repair. These processes are regulated by several genes, including p53 and members of the Bcl-2 family such as Bax and Bcl-2(Bowen et al., 2006; Elmore, 2007).

The Bax gene is a proliferative suppressor gene that encodes Bax protein which promotes apoptosis. Bax is a protein that forms an isomeric dimmer with Bcl-2. It counteracts the action of Bcl-2 in the presence of various cell death signals and induces apoptosis(Prabhu et al., 2012). Bax protein expression has been identified in various human tissues such as the prostate, colon, breast, testis and ovary. But, little is known about Bax protein expression in gastric injury.(Qiao et al., 2011)reported that high level expression of Bax protein was expressed at early stage of gastric ischemia reperfusion of rats, and decreased gradually after that. This result showed that down-regulated of Bax protein related to healing effect of gastric ulcer.

2.12. Phytochemical compounds

Phytochemicals are non nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytoochemicals such as phenolic acids, flavinoids, lignans, anthocyanins, flavonols, catechins, procyanidins, flavanols and many more (Heneman & Zidenberg-Cherr, 2008). All of these compounds play different roles based on their chemical structure. For example, carotinoids in carots, flavonoids in fruit and vegetables, polyphenols in tea and allyl sulfides in onions play as an antioxidant agent. In this action, they protect the cells oxidative damage and reduce the risk of developing certain types of cancer (Edeoga et al., 2005).

Moreover, phytochemicals including indols which can be found in cabbage and terpenes in citrus fruits can act to stimulate the enzymes which can help in reducing the risk for breast cancer. Saponin which is found in beans also helps to disturb the DNA replication which thereby prevents the multiplication of cancer cells. Phytochemicals in the group of allician coming from garlic has bacterial properties which can act as the antibacterial agent (Lacikova et al., 2009).

Other study also showed that phenolic compounds, including their subcategory, flavonoids commenly found in nuts, olive oils, vegetables and fruits have effects on thrombosis and tumorogenesis. Apart from that, phytoestrogenes which can be found in soy and also other flaxseed oil, whol grains, fruit and vegetables also have the antioxidant properties. According to some study conducted in United states, they provide that phytoestrogenes gave positive effects on the animal cell culture models of cancer (El-Sawi et al., 2008).

CHAPTER III

MATERIALS AND METHODS

3.1. Plant specimen and extract preparation

Leaves of the plants were purchased from Ethno Resources Sdn Bhd. Selangor, Malaysia, and taken to University Putra Malaysia, Selangor to be identified by comparison with the Voucher specimen of *Jasminum Sambac* and *Hemigraphis Colorata* deposited at the Agricultural Conservatory Park, Malaysia. Leaves were dried and blended by using electrical blender till fine powder. Powder of 100 grams was mixed five hundred millilitre ethanol (95%) for 72 hours than fine muslin and filter paper used to filter the mixer. Eyela evaporator (Rotary, Sigma-Aldrich, USA) reduced pressure used to yield filtered powder and yielded approximately 11.3% (*J.Sambac*) and 9.75% (*H.Colorata*).

3.2. Wound Healing Experiment,

Normal saline used as solvent to mix extract and gum acacia(vehicle) as described by (Shetty et al., 2008).Gum acacia (2 grams) was soaked in normal saline of hundred millilitre. Gum acacia of 200mg in 10 mL of solution used to mix up with one gram and two grams from each plant extract each. So, 1 mL of each solution contains 100 and 200 mg of extract, respectively (100 mg/mL = 20 mg/0.2 mL and 200 mg/mL = 40 mg/0.2 mL).

3.3. Antiulcer Experiment

Carboxymethyl cellulose (CMC) was used to dissolve the plant extracts and prepared them according to the designed doses 50, 100, 200, 300 and 400 mg/kg body weight for *H. colorata* and of 62.5, 125, 250, and 500 mg/kg body weight (5 mL/kg body weight) for *J. Sambac* to be given to the animals by oral administration (Mahmood A Abdulla et al., 2009).

Table 3.1 Investigated Medicinal plants details and botanical name

Latin name	**Local name**	**Family**	**Part tested**	**Traditional uses**
Jasminum Sambac. Linn	melur	Oleaceae	Leaves	Fever, Acne, eye swollen eye, headache, breast cancer, skin inflammation and Antimicrobial
Hemigraphis Colorata (Blume)	Red Ivy	Acanthaceae	Leaves	skin wounds and abscesses, Antibacterial, Diabetic

3.4. Antioxidants Activity of Plants Extracts

The first part of this experiment was established to evaluate the antioxidant power of *J. sambac* and *H. colorata* crude extracts using ferric reducing antioxidant power assay (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total phenolic contents.

3.4.1. Ferric Reducing Antioxidant Power Assay (FRAP)

The ferric reducing activity of the plant extracts were evaluated using the method developed by (Benzie & Strain, 1996). The reaction mixture 46 contained 300 mmol/l acetate buffer, 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mmol/L of HCl acid and 20 mmol/L of FeCl3. 6H2O. FRAP reagent prepared by mixing 25 ml of acetate buffer, 2.5 mL of TPTZ solution and 2.5 ml of FeCl3. 6H2O. Then it was incubated at 37°C in water bath for a period of 5 minutes. Adjusted a blank reader at 593nm performed in spectrophotometrically. After that, 30 µl of extract or standard and 90 µl of distilled water were added to 900 µl of active FRAP reagent. Absorbance was taken at 0 minute immediately after mixing with active FRAP reagent. Following, reading at 4 minutes and standard curve plotted as per standard solution used (FeSO4) .Results were calculated as per standard unit µmol Fe(II)/mg dry weight of crude extract (appendix II).

3.4.2. The Scavenging Activity of DPPH

The crude extracts activities to scavenge DPPH free radical were determined spectrophotometrically and calculated as % DPPH inhibition. DPPH acquired proton from the sample and this appear as reduction in intensity of sample from dark violet to

light yellow. Antioxidant compound donating electron (proton) and this reduce DPPH radical this indicated in intensity reduce in sample concentration to light yellow. This test was done according to the procedure mentioned by (Gerhauser,et al.,2003). 5 µl (sample) mixed with ethanol than added into 195µl from 100 µM DPPH reagent in ethanol and mixed in 96 micro plate. The concentration of sample was taken over 3 hours with interval of every 20 mins at 515nm.(Appendix II)

Equation: DPPH radical scavenged (%) = (OD blank – OD sample) / (OD blank) × 100%

3.4.3 Total Phenolic Content (TPC)

The *J.Sambac* and *H.Colorata* extracts were measured for their total phenolic content by using Folin-Ciocalteu reagent and were calculated as values in mg (GAE)/g equivalent to gallic acid according to Folin-Denis colorimetric method (AOAC, 1995).(AppendixII)

3.5 Acute Toxicity

There essential points to be considered during acute toxicity studies. All animals should be healthy prior conducting a study in acute toxicity. Normal animals are suspected to be physical and biological stable as human being metabolism. This will show up different respond within a group of species during the test timeframe. Metabolism and pharmacokinetics respond differ from animal to another as happen in human. Experimental animals must match the criteria for acute toxicity such as: animal species, age, sex, and size of treatment. Is difficult to match animal's metabolism trial to human but is important to select healthy with perfect physiology. Animals were selected between 6-8 weeks old because is recommended for acute toxicity study. Equal number of male and female selected in order to study all aspects such as physical behaviour,

Serum biochemistry (Liver Function Test, Renal Function Test) and their histology manifest. Adequate experimental animals were selected to obtain significant data for analysis. Ethical should be obtained prior conducting animals study including, diet, dose administration, housing and healthcare.

The acute toxic study aimed to determine safe dose from each plant extracts (*J. Sambac* and *H. Colorata).*For each plant extract, Rats were differentiated into three separate groups, each group contains six rats. Group 1 administered distilled water served as control. Group 2 and Group 3 orally administered plant extracts 2 and 5g/kg, single dose, respectively.

After treatment pellet (food) withheld for 3 to 4 hours. Animals were observed 30 min then 2,4,24 and 48 hrs for any clinical symptoms and toxicological sickness. Food was withheld for a further three to four hours after treatment. Over two weeks to observe any mortality and kept fast overnight. On day 15^{th} animals were sacrificed. Samples were taken for biochemistry and histology evaluation. The ethics was approved by the animal house committee, Faculty of Medicine, University of Malaya, (Ethic No. PM/07/05/2008/MAA (a) (R). All experimental animals maintained according to “Care and Laboratory Animals guidelines” provided by the national Institute of health and prepared by the Academy of Sciences. (Fig. 3.1).

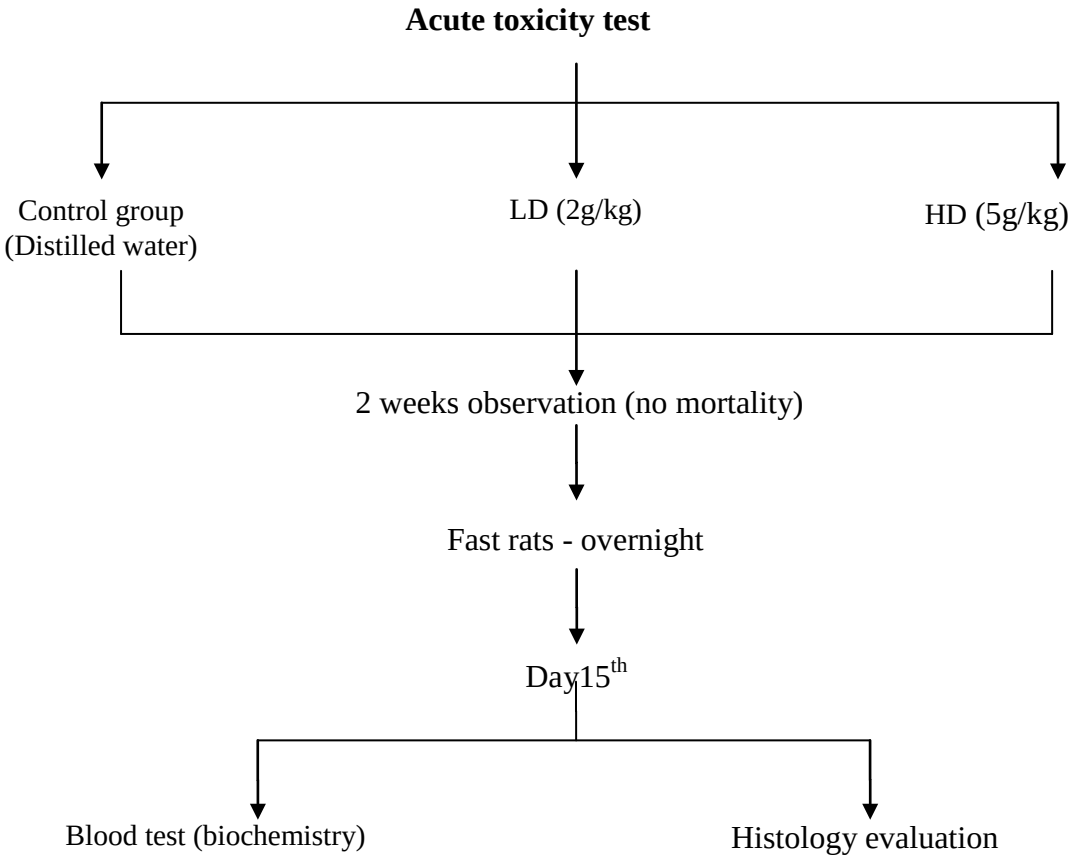


Fig. 3.1. Flow diagram of acute toxicity study.

3.6. Wound Healing Activity of the *J. sambac* and *H. colorata* Crude Extracts

3.6.1. Intrasite

Intrasite gel was obtained from the Medical Centre, University of Malaya. Intrasite gel is transparent visible and clearly aqueous gel, contains of modified 2.3% carboxymethylcellulose polymer along with propylene glycol (20%) as a humectants moisture and act as preservative. Fast absorb with wound dressing access exudates and produce moisture enviroment without cause any maceration in tissue.Intrasite gel helps to rehydrate necrotic tissue and suitable for all wound stages such as moist wound management,granulating,epithelising,and wound closures stages.It is non adherence agents and does not cause harm skin around the wound.This has ideal potential for all wound management stages.Trade made for smith and Nephew Healthcare Limited(williams,1994) (Williams, 1994).

3.6.2. Animal Experiment

Male rats of species *Sprague Dawley* purchased from the animal house that belongs to Faculty of Medicine, University of Malaya and the ethics were approved by the committee of animal house at Faculty of Medicine, University of Malaya PM/28/09/2006/111/MAA (R). For each plant, animals were isolated randomly into four groups of six rats per group. In addition, the rat that weighed between 220 - 250 g weight was caged individually. All animals were kept under human care and given water under human care.

3.6.3. Wound Excision in Experimental Rats

The animals anesthetized entirely by using ketamine im injection (30mg/kg, 100mg/ml) with 0.09mL and 0.01mL xylazil im injection (3mg/kg, 100mg/mL).The skin clipped by electronic clipper, disinfected with 70% alcohol. An area of uniform diameter of 2.00 cm (circular area = 3.14 $cm^{2)}$ with the aid of circular seal. All animals were excised from the dorsal neck nape as described previously (M.A. Abdulla et al., 2009) (Figure 3.2). Animal's incision was done carefully to avoid muscle incision. After the operative process the entire wound was open and measured direct by placing transparent paper. The transparent paper was immediately tracing it out and placed on $1mm^2$ sheet and traced it out. Area was calculated, as described previously (Al-Bayaty & Abdulla, 2012).

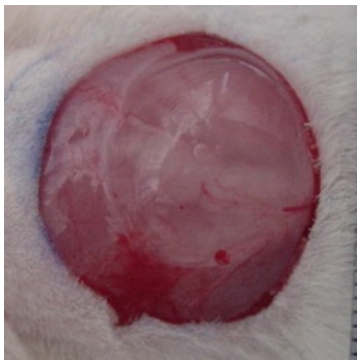


Figure 3.2**:** Excision skin with 2 cm diameter taken on Day 0, before topical application.

3.6.4. Topical Wound Application

The rats of Placebo control wound (Group 1) were treated twice daily with 0.2 mL gum acacia in normal saline (20 mg/ml) (M.A. Abdulla et al., 2010). Wounds of Group

2 rats are reference standard control dressed topically with Intrasite gel 0.2 ml, 2 times a day. Ethanol plant extracts of each plant *(J.Sambac* and *H.Colorata)* of 0.2 mL (100 and 200 mg/mL) in vehicle each were dressed topically two times daily to Group 3 and 4 wound, respectively.

3.6.5. Wound Closure Assessment

The wound closure area was assessed and calculated in per mm^2. Wound traced on Day 0, 5 and 10 post wounding and calculation was given per percentage of healing area to correlate with post wounding day where transparent sheet used and marker pen in completely anaesthesia as described in previous studies (M.A. Abdulla et al., 2010). The areas of wound were analyzed and recorded by using a graph sheet. The percent grade of each group on wounds healing days was determined (Abdulla et al., 2010).

3.6.6. Histopathology Evaluation in Wound Closure

Samples collected from the area of wound in each experimental rat were fixed in buffered formalin 10%. Tissue processing was performed by fixation in Bouin's fluid, dehydration, embedding, sectioning, and staining. Hematoxylin and Eosin used to assess basic histological determination (Behmer et al., 1976a). Collagen was evaluated by using Masson's trichrome-stained method. Stained sections were evaluated by using light microscope to observe morphology of fibroblasts, collagen deposition, angiogenesis and epithelisation.

3.6.7. Oxidative Damage Evaluation from the Granulation Tissue

Post wounding tissues (granulation tissue) were collected on day 10^{th} for oxidative damage evaluation activity Lipid peroxidation (MDA) and antioxidants enzymes.

3.6.7.1 Protein Determination

Protein content was determined from wound tissue of each animal as described by (Bradford, 1976). In summary, wound granulation tissues was homogenized in 1.15% $CaCl_2$ (1:5 (w/v).Homogenate mixer of 0.1 mL added to Bradford reagent. The reading was taken at 595nm parallel to buffer (phosphate,pH 7.4).Bovin serum albumin (BSA) used as standard and treated similarly at concentrations of 0,…,40,…,80 and 100µg/mL in phosphate buffer,pH7.4.

3.6.7.2. Superoxide Dismutase (SOD) Evaluation

SOD was evaluated by using Superoxide Dismutase Assay Kit (Catalog no. 706002, from Cayman Chemical Company) (Appendix I). Fig 3.3 shows the assay:

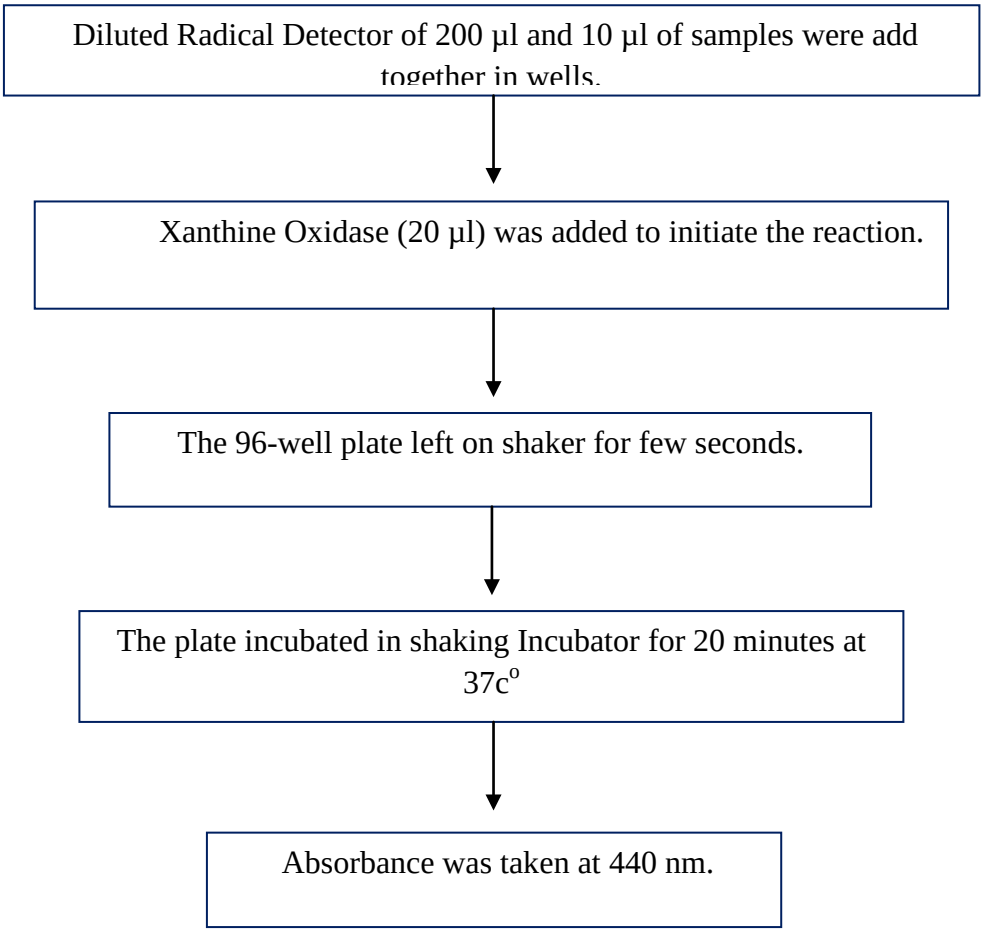


Fig. 3.3 Superoxide dismutase (SOD) assay

3.6.7.3. Glutathione (GPx) Assay

GPx was performed by using Glutathione Peroxidase Assay Kit (Catalog no. 703102, from Cayman Chemical Company). (Appendix II).

3.6.7.4. Malondialdehyde (MDA) Assay

Post wounding tissue homogenate on day 10^{th} was taken for malondialdehyde evaluation to determine lipid peroxidation level using TBARS Assay Kit (catalogue no.10009055) (Appendix.II)

3.7. Gastroprotective Activity of *J. sambac* and *H. colorata* Crude Extracts

3.7.1. Omeprazole

Omeprazole was purchased from Medical Centre, Universiti Malaya. It Is used as a standard reference antiulcer medicine and soluble in 0.5% w/v carboxymethyl cellulose (CMC). It was Orally administered (20 mg/kg), as described (Mahmood, Mariod, et al., 2010) .

3.7.2. Gastric Ulcer Induction in Animals by HCl/Ethanol

Adult male *Sprague Dawley* rats having weights between 200-225g were provided by the animal house of Faculty of Medicine (Ethic No. PM/28/09/2006/111/MAA(R). The experimental animals were separated to 15 groups, each group consist of 6 rats and individual rat per cage. Standard pellet diets provided and tap water in each cage.

Twenty four hours before the experiment, all rats were kept fasted overnight without access to food except water was accessible till before experiment procedure by 120 minutes (Mahmood, Mariod, et al., 2010). Gastric induction model described by Mizui and Doteuchi (Mizui & Doteuchi, 1986) using 150 mM Hcl / absolute ehanol solution 40:60 v/v for mucosa injury. Controls groups (Normal and Ulcer) were given CMC by oral administration. An amount of 20mg/kg omeprazole was give orally to the reference drug group. The animals of experimental groups were administered orally with the plant leaves extract of *H.Colorata* in the doses 50,100,200,300 and 400 mg/kg and the plant leaves extract of *J. Sambac* in the doses 62.5,125,250, and 500 mg/kg. Sixty minutes later, normal animals group were orally feeded with CMC, whereas the reference group, ulcer group and experimental groups were orally adminstered 5mL/kg of Hcl/ethanol to induce gastric ulcer. Sixty minutes later, all the rats were anesthetized with over dose of xylazine and ketamine, immedialetly the procedure of stomach excision performed.

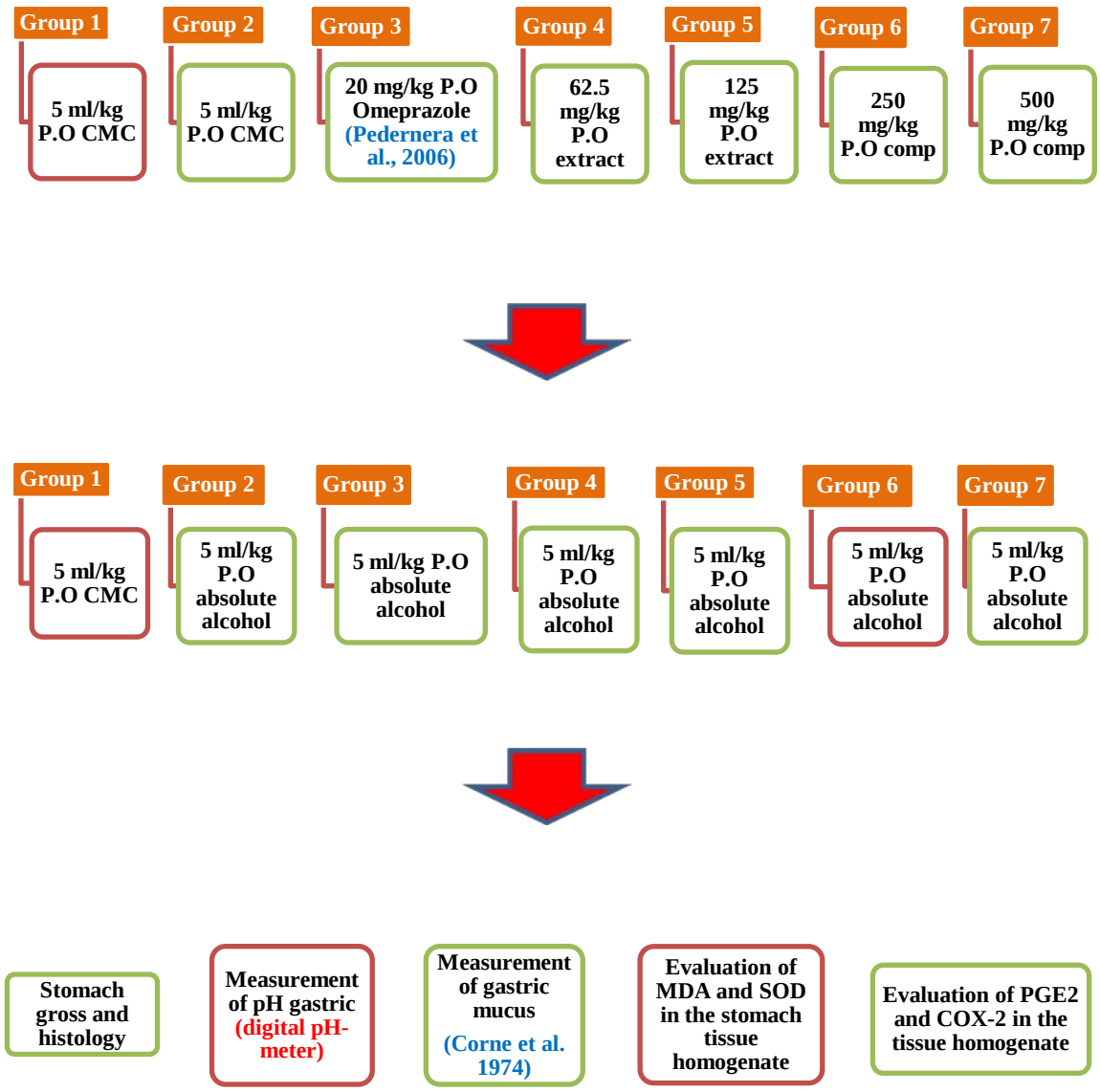


Fig. 3.4: Anti-ulcer experimental design of *J. sambac* (Mota et al., 2008)

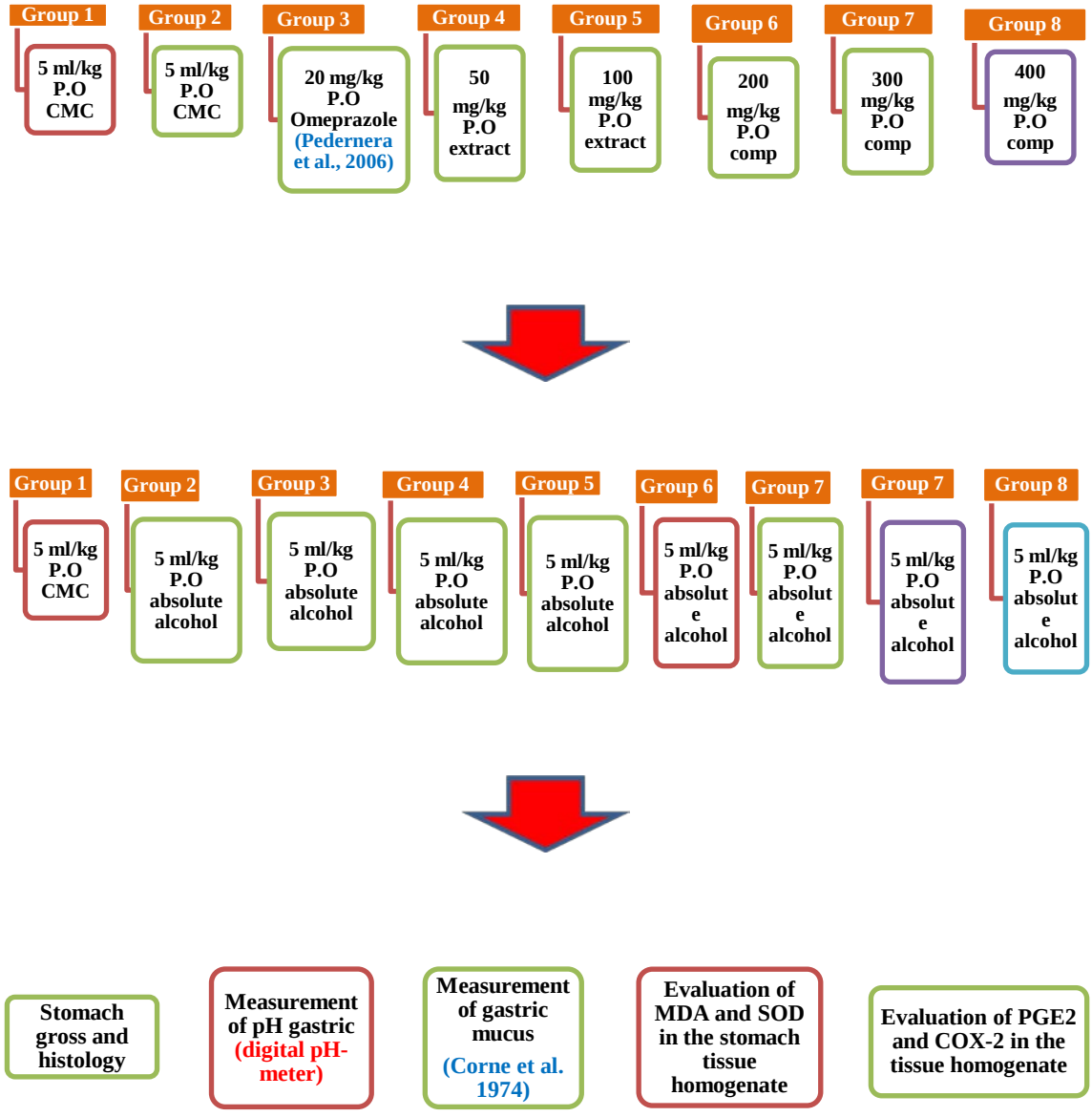


Fig. 3.5: Anti-ulcer experimental design of *H. colorata* (Amr & Maysa, 2010)

3.7.3. Measurement of pH

Following antiulcer experiments as explained in section 3.7.2, an overdose of ketamine (30mg/kg, 100 mg/mL) and xylazyl (3mg/kg,100 mg/mL) in a ratio of 4:1 v/v was given intramuscularly to all the animals to sacrifice them and Stomach excision process was performed immediately. Collection of gastric juice was performed and spinned at 3500 rpm for 10 min then gastric pH was read by using digital pH meter.

3.7.4. Assessment of Gastric Wall Mucus

The procedure described by Corne *et al* and Al-Qarawi *et al* (Corne *et al.*, 1974; Al-Qarawi *et al.*, 2005) used to determine the gastric wall mucus for all experimental animals. Following anti ulcer experiments, the stomachs of animals were excised, properly washed and the gastric contents were removed through an opening made along the greater curvature of the stomach. Carefully labeled tubes containing 10 mL of 0.1% Alcian blue solution were prepared to incubate the glandular part of each stomach individually after removal and weighing for 2 hrs. After incubation, the excess dye was removed by washing twice with 10 mL of 0.25 M sucrose. An amount of 10 mL of 0.5 M magnesium chloride was used to extract each stomach by random shaking for 1 min at 30 min intervals for 2 hrs. The resulting solutions were mixed with equal volumes of diethyl ether and vigorously shacked. The emulsions produced from shaking were then centrifuged at 3000 rpm for 10 mins. The standards were prepared from different concentrations of 0.1% stock solution of Alcian blue. The aqueous layer was then assayed against a buffer blank and the reading of the spectrophotometer was recorded at 580 nm. The quantity of blue dye recovered per gram of wet glandular tissue was then calculated using the equation of the standard curve.

3.7.5. Gastric Lesion Evaluation

Gastric ulcer physical observed elongated bands with marked haemorrhage lesion parallel to axis longitude of the stomach. The gastric mucosa of each experimental animal was assayed for any mucosal damage using planimeter (10×10 mm^2 = ulcer area) under (1.8×) microscope. The lesion of the gastric ulcer was measured by calculating squares (2mm by 2mm), each occupied by ulcer band. Total number of lesion areas calculated to determine ulcer area (UA),the sum × 4 ×1.8 = UA (mm^2) was recommended by (Bardi et al., 2011). Inhibition percentage (1%) was calculated, according of (M. A. Abdulla et al., 2010).

$$(I\%) = [(UA_{control} - UA_{treated}) \div UA_{control}] \times 100\%$$

3.7.6. Histopathological Examination of the Gastric Mucosa

Histological examination was performed after the evaluation of ulcer lesion. A concentration of 10% formalin solution was used to fix stomach tissue sections and the tissue processing (Dehydration, Cleaning and Infiltration) was done automatically using Automated Tissue Processing Leica (TP1020). Paraffin wax embedding of the tissue sections was done in Leica HISTOEMBEDDER and the embedded tissues were sectioned using a microtome to produce 5 μm paraffin wax tissue sections. Then, the sections were stained with Haematoxylin & Eosin followed by mounting with DPX mounting media. Next, the mounted sections were evaluated for microscopic examination using light microscope (Carl Zeiss, Japan). All the histological techniques were explained in details in appendix I. (Behmer et al., 1976).

3.7.7. Immunohistochemistry

Using poly-L-lysine-coated slides, liver sections were prepared and heated in an oven (Venticell, MMM, Einrichtungen, Germany) for 25 minutes at 60^{o}C. After heating, xylene was used to deparaffinize and graded alcohol to rehydrate stomach sections. A concentration of 10 mM sodium citrate buffer was used for antigen retrieval. Immunohistochemistry protocol was performed following the procedure explained in the manual's instructions (DakoCytomation, USA). In brief, hydrogen peroxide sodium azide of concentration 0.03% was used to block the endogenous peroxidase for five min followed by careful washing of the tissue sections using wash buffer followed by incubation with the provided primary antibodies Bcl-2–associated X protein (Bax) (1:500) and heat shock protein (Hsp-70) (1:100) (Santa Cruz Biotechnology Inc, California, USA) for 15 minutes. Following re-washing, Streptavidin-HRP was then added and the sections left incubated for 15 min followed by washing. The chromagen diaminobenzidine-substrate was added to the sections and left for 10 min incubation followed by washing and hematoxylin counterstaining for five seconds. Weak ammonia (0.037 mol/L) was used in dipping the tissue sections which are finally washed and cover slipped to be examined using light microscopy. Positive antigens were brown stained under the microscope.

3.7.8. Oxidative Damage Evaluation from the Gastric Tissue Homogenate

3.7.8.1. Preparations of samples

For assaying PGE_2, SOD and MDA in the gastric tissue of all ainmal groups, small pieces of gastric tissues were and weighed (about 200 mg for each) (Yildirim et al., 2007). The tissues were homogenized in Teflon homogenizer (Polytron, Heidolph RZR

1, Germany) was used to homogenize the tissues using phophate buffer saline. After centrifugation at 18,000 x g for 15 min at 4°C, the supernatant was collected and divided into aliquots.

3.7.8.2. Superoxide Dismutase (SOD) Activity assessment

The activity of SOD was measured using the protocol provided by Cayman's Superoxide Dismutase Assay Kit, Item Number 706002,USA. (Appendix).

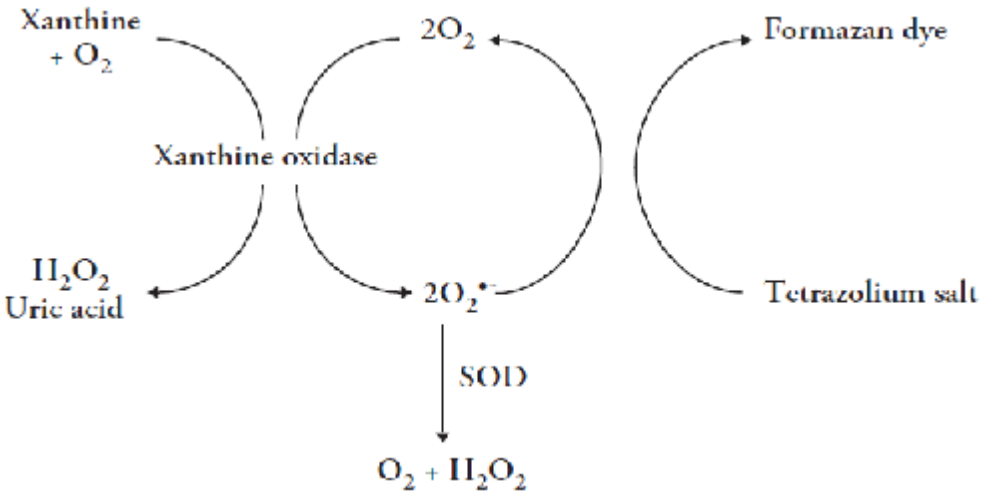


Fig. 3.6 Diagram shows the reaction of the superoxide dismutase assay

3.7.8.3. Membrane Lipid Peroxidation (MDA) Evaluation

The degree of lipid peroxidation in the mucous membrane of the stomach was estimated by level of malondialdehyde (MDA) using Cayman TBARS Kit (catalogue no.10009055).Details in Appendix II.

3.7.8.4. Measurement of PGE2 level

The level of prostaglandin E2 (PGE2) in the stomach tissue homogenate was measured by following the instructions explained in Prostaglandin E2 EIA Kit-Monoclonal Cayman Chemical (Item Number 514010), USA. The details are explained in Appendix II.

3.7.8.5. Cyclogenase-2 (COX-2) Assay

COX-2 was assayed in the stomach tissue homogenate by using immunoassay Elisa kit Cat. No.E0699Ra (Uscn Life Sciences Inc. China) (Appendix II).

3.8. Isolation of the β-sitosterol from *J. sambac* and Octyl gallate from *H. colorata* Crude Extracts

3.8.1. Instrumentation

Column Chromatography

Agilent XDB C-18 Reversed phased column, dimensions 4.6 x 250 mm and Particle size, 5.0 μm.

HPLC Components for J. sambac

Equipment: HPLC Agilent 1100 Series equip with auto sampler and fraction collector.

Column: Agilent Zorbax ODS C-18 (4.6 X 250 mm, 5.0 mm, 70A)

Volume of injection: 10 μl

Solvents: A: Acetonitrile **,** B: MilliQ Water

Flow rate: 1.0 ml/ min

Post run: 2 minutes

Table 3.2: HPLC gradient conditions of *J. sambac*

Time	A%	B%
0.01	50	50
10	100	0
30	100	0
32	0	100

HPLC components for H. colorata

Instrument: Agilent 1200 Series HPLC system with Capillary pump and degasser, micro-well plate sampler with thermostat, and Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source.

LC Parameters:

Column Used: Agilent Zorbax SB-C18 (0.5 x 150 mm, 5um). Part no: 5064-8256

Flow Rate: 18uL/min from Agilent 1200 series capillary pump (micro flow)

Solvents:

0.1% formic acid in water (A); 90% Acetonitrile in water with 0.1% formic acid (B)

Injection Volume: 1Ul

Table 3.3: HPLC gradient conditions of *H.colorata*

Time	B%
Initial	10
20	100
30	100

LCMS components

Instrument: Agilent 1200 Series HPLC system with Capillary pump and degasser, micro-well plate sampler with thermostat, and Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source.

LC Parameters:

Column Used: Agilent Zorbax SB-C18 (0.5 x 150 mm, 5um). Part no: 5064-8256

Flow Rate: 18uL/min from Agilent 1200 series capillary pump (micro flow)

Solvents: Acetonitrile +0.1% formic acid

Note: Same solvents were used for both Postive and Negative polarity.

Injection Volume: 1uL

Sample Analysis: Isocratic run with Acetonitrile +0.1% Formic acid for 30 minutes

MS Parameters:

Ion Polarity: Positive & Negative

Vcap: 3500V

Fragmentor Voltage: 125V

Skimmer: 65 V

OCT 1 RF Vpp: 750 V

Drying Gas: 5L/ min

Gas Temperature: 300°C

Nebulizer: 30 psig

Ref nebulizer: 1 psig

Data Acquisition: MS only mode. For Positive Polarity: range 103-1500 m/z

For Negative Polarity: Range 115-1000 m/z

3.5.2. Isolation of β-sitosterol from *J.sambac* Petroleum Ether Crude Extract

An amount of 0.61 g of *J. sambac* Petroleum ether crude sample was dissolved in 2 ml acetone and mixed with 1.2 g of silica and dry-packed using rotary evaporator to form powder sample. The powdered sample was subjected to column chromatography (CC) containing 36 g silica gel. The fractionation step was based on gradient elution method and the solvent system used was hexane- acetone (Fisher Scientific AR Grade, UK). A total of 14 fractions afforded. A Thin Layer Chromatography (TLC) (Merck, Silica gel 60 F254, Japan) experiment of the 14 fractions versus β-sitosterol standard was carried out (Fig. 3-7). Fractions were combined to give 4 fractions labeled as JS-1-5, JS-6-8 and JS-9-10 and JS-11-14. Fractions 6-8 (JS-6-8) containing beta-sitosterol (*Rf* 0.36) was dried using rotary evaporator and weighed using analytical balance. JS-6-8 (0.51 mg) was re-purified using column chromatography (CC) containing 36 g silica gel and petroleum ether-chloroform solvent system (Fisher Scientific AR Grade, UK). A total of 16 sub-fractions afforded and were labeled as JS-6-8-1 to JS-6-8-16. TLC profile of the sub-fractions (JS-6-8-1 to JS-6-8-16) versus the pure standard was carried out. JS6-8-11 was further purified using high performance liquid Chromatography (HPLC) and the purified compound was determined by LCMS using METLIN DATABASE (Appendix II).

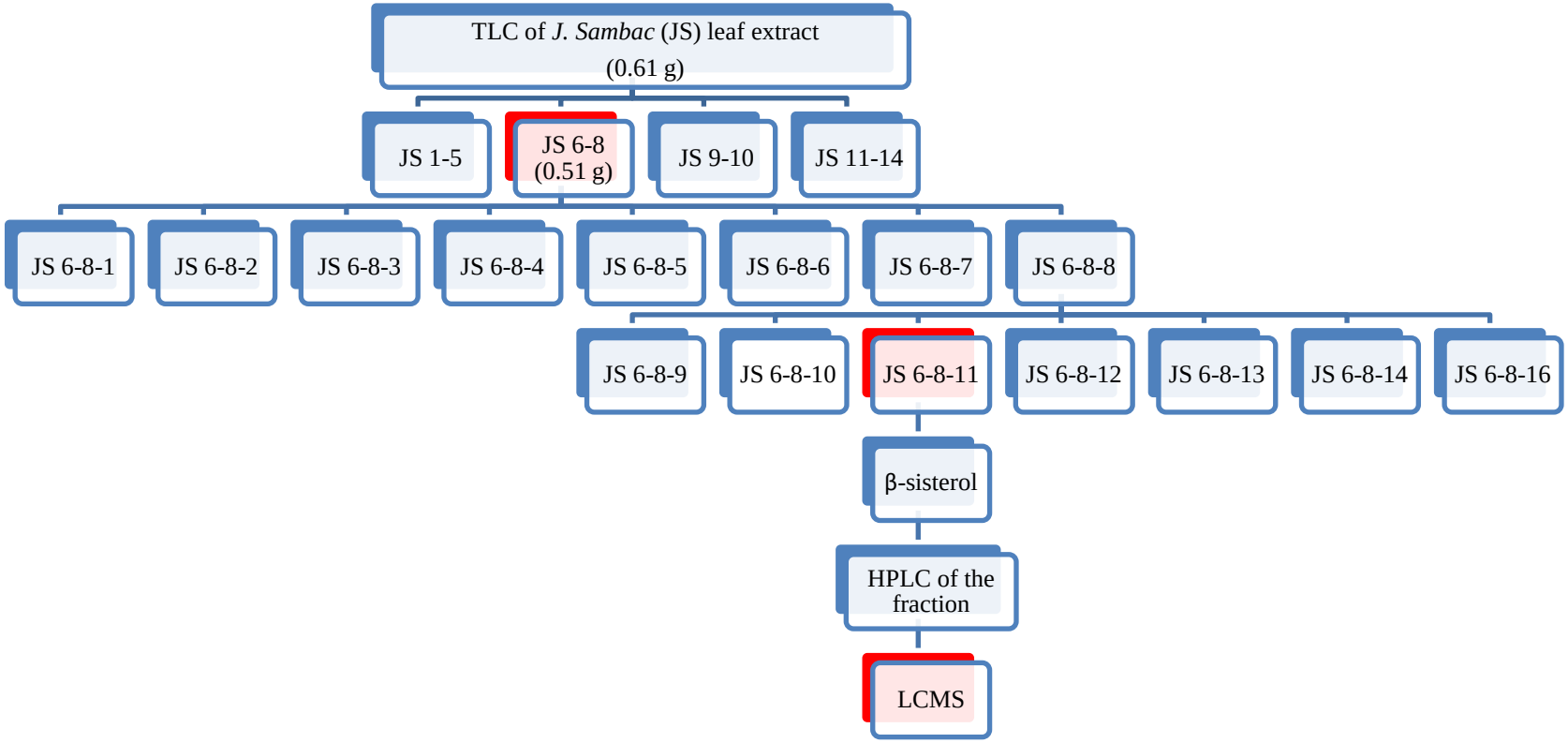


Fig. 3.7: Fractionation of *J. sambac* petroleum ether crude extract

3.8.3. Isolation of Octyl Gallate from *H. colorata* Petroleum Ether Crude Extract

A quantity of 112.6 mg of *H. colorata* petroleum ether crude sample was dissolved in 2 ml acetone. The sample solution was mixed with 225 mg of silica and dry-packed using rotary evaporator to form powder sample. The powdered sample was subjected to column chromatography (CC) containing 33 g silica gel. The fractionation step was based on gradient elution and the solvent system used was hexane- acetone (Fisher Scientific AR Grade, UK). A total of 4 fractions afforded from column chromatography fractionation (Fig. 3-8). Thin Layer Chromatography (TLC) (Silica gel 60 F254, Merck, Japan) experiment of the 4 fractions versus Octyl gallate standard was carried out using 70 Hexane: 30 acetone solvent. The TLC profile was monitored under UV light at 254 nm. All the fractions were dried using rotary evaporator and weighed using analytical balance. HPLC of fraction HC-4 versus Octyl gallate standard was performed and the compound was identified by LCMS using METLIN DATABASE (Appendix II).

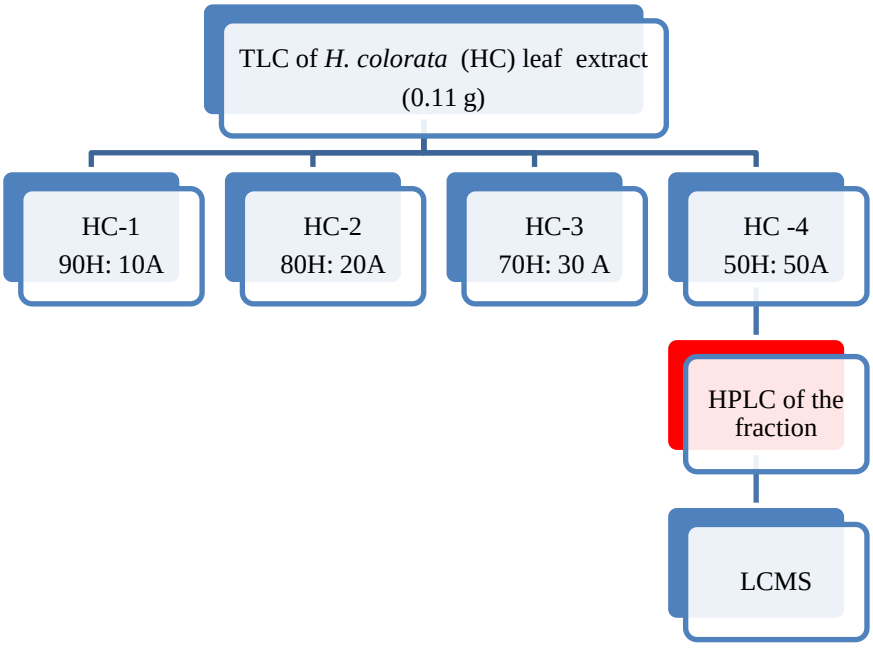


Fig. 3-8: Fractionation of *H. colorata* petroleum ether crude extract

3.9. *In Vivo* Gastroprotective Activity of β-sitosterol and Octyl gallate against HCL/Ethanol-induced Gastric Ulcer in Rats

3.9.1. Animal Experiment

Adult male *Sprague Dawley* rats having weights between 200-225g were provided by the animal house of Faculty of Medicine (Ethic No. PM/28/09/2006/111/MAA(R). The experimental animals were separated to 7 groups, each group consist of 6 rats and individual rat per cage. Standard pellet diets provided and tap water in each cage. Twenty four hours before the experiment, all rats were kept fasted overnight without access to food except water was accessible till before experiment procedure by 120 minutes (Mahmood, Mariod, et al., 2010). Gastric induction model described by Mizui and Doteuchi (Mizui & Doteuchi, 1986) using 150 mM Hcl / absolute ehanol solution 40:60 v/v for mucosa injury. Controls groups (Normal and Ulcer) were given CMC by oral administration. An amount of 20mg/kg omeprazole was give orally to the reference drug group. Animals of the experimental groups were administered orally with β-sitosterol and octyl gallate compounds in the doses 5 and 10 mg/kg for each compound separately. Sixty minutes later, normal animals group were orally feeded with CMC, whereas the reference group, ulcer group and experimental groups were orally adminstered 5mL/kg of Hcl/ethanol to induce gastric ulcer. Sixty minutes later, all the rats were anesthetized with over dose of xylazine and ketamine, immedialetly the procedure of stomach excision performed.

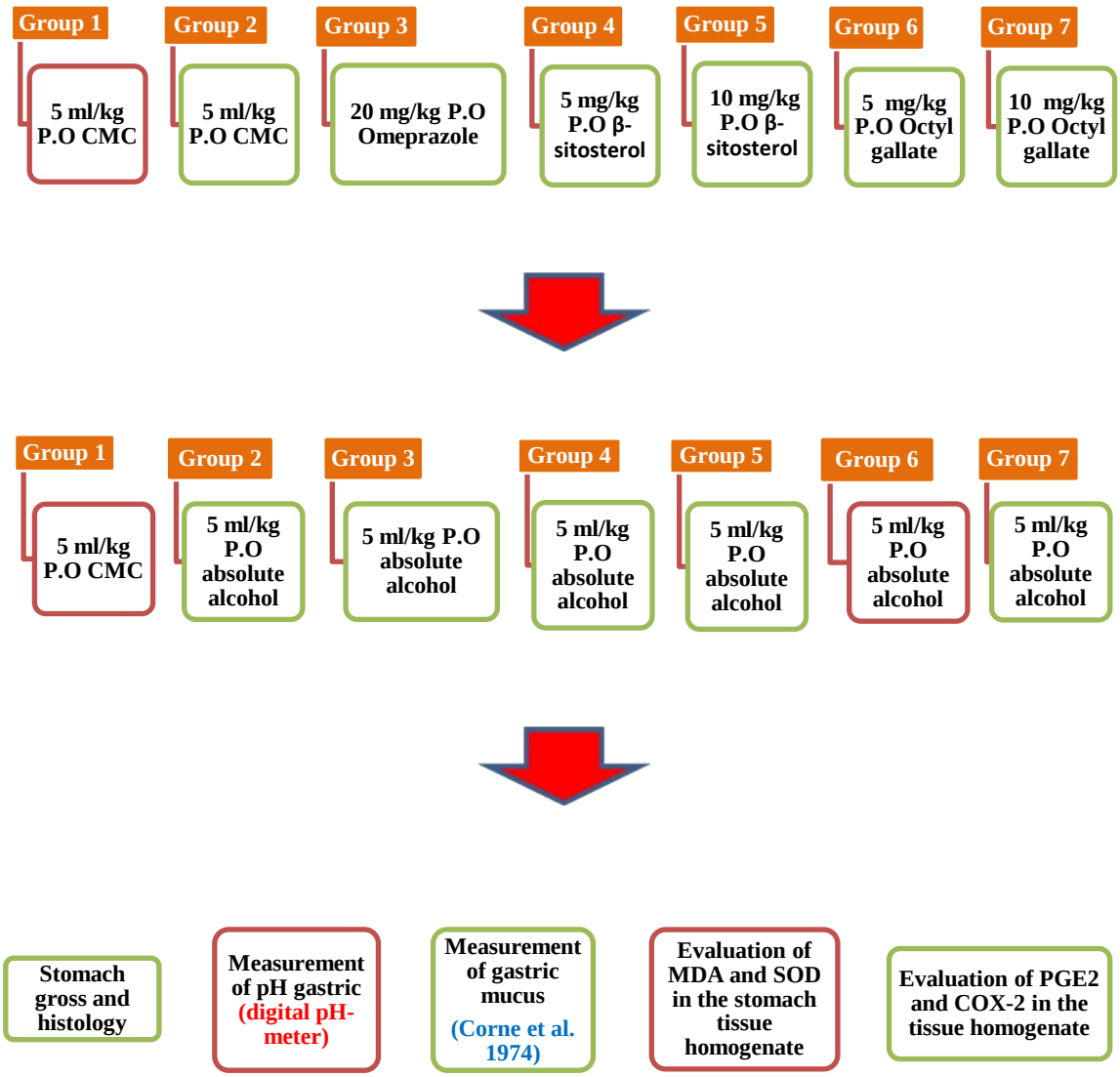


Fig. 3.9: Anti-ulcer experimental design of β-sitosterol and Octyl gallate compounds

3.9.2. Measurement of pH

Following antiulcer experiments as explained in section 3.4.2 the animals were sacrificed by overdose of ketamine (100 mg/mL) and xylazine (100 mg/mL) in a ratio of 4:1 v/v intramuscularly. The stomachs of all experimental rats were excised and the gastric contents were collected and centrifuged at 3500 rpm for 10 mins to determine gastric pH by using electronic pH meter Model W-500.

3.9.3. Gastric Wall Mucus

The procedure described by Corne *et al* and Al-Qarawi *et al* (Corne *et al.*, 1974; Al-Qarawi *et al.*, 2005) used to determine the gastric wall mucus for all experimental animals. Following anti ulcer experiments, the stomachs of animals were excised, properly washed and the gastric contents were removed through an opening made along the greater curvature of the stomach. Carefully labeled tubes containing 10 mL of 0.1% Alcian blue solution were prepared to incubate the glandular part of each stomach individually after removal and weighing for 2 hrs. After incubation, the excess dye was removed by washing twice with 10 mL of 0.25 M sucrose. An amount of 10 mL of 0.5 M magnesium chloride was used to extract each stomach by random shaking for 1 min at 30 min intervals for 2 hrs. The resulting solutions were mixed with equal volumes of diethyl ether and vigorously shacked. The emulsions produced from shaking were then centrifuged at 3000 rpm for 10 mins. The standards were prepared from different concentrations of 0.1% stock solution of Alcian blue. The aqueous layer was then assayed against a buffer blank and the reading of the spectrophotometer was recorded at 580 nm. The quantity of blue dye recovered per gram of wet glandular tissue was then calculated using the equation of the standard curve.

3.9.4. Evaluation of Macroscopic Gastric Lesion

Gastric ulcer physical observed elongated bands with marked haemorrhage lesion parallel to axis longitude of the stomach. The gastric mucosa of each experimental animal was assayed for any mucosal damage using planimeter (10×10 mm^2 = ulcer area) under ($1.8\times$) microscope. The lesion of the gastric ulcer was measured by calculating squares

(2mm by 2mm), each occupied by ulcer band. Total number of lesion areas calculated to determine ulcer area (UA),the sum × 4 ×1.8 = UA (mm^2) was recommended by (Bardi et al., 2011). Inhibition percentage (1%) was calculated, according of (M. A. Abdulla et al., 2010).

$$(I\%) = [(UA_{control} - UA_{treated}) \div UA_{control}] \times 100\%$$

3.9.5. Histopathological Examination of the Gastric Mucosa

Histological examination was performed after the evaluation of ulcer lesion. A concentration of 10% formalin solution was used to fix stomach tissue sections and the tissue processing was done automatically using Automated Tissue Processing. Paraffin wax embedding of the tissue sections was done and the embedded tissues were sectioned using a microtome to produce 5 μm paraffin wax tissue sections. Then, the sections were stained with Haematoxylin & Eosin followed by mounting with DPX mounting media. Next, the mounted sections were evaluated for microscopic examination using light microscope (Carl Zeiss, Japan). All the histological techniques were explained in details in appendix I. (Behmer et al., 1976).

3.9.6. Immunohistochemical Staining

Using poly-L-lysine-coated slides, liver sections were prepared and heated in an oven (Venticell, MMM, Einrichtungen, Germany) for 25 minutes at 60^{o}C. After heating, xylene was used to deparaffinize and graded alcohol to rehydrate stomach sections. A concentration of 10 mM sodium citrate buffer was used for antigen retrieval. Immunohistochemistry protocol was performed following the procedure explained in the

manual's instructions (DakoCytomation, USA). In brief, hydrogen peroxide sodium azide of concentration 0.03% was used to block the endogenous peroxidase for five min followed by careful washing of the tissue sections using wash buffer followed by incubation with the provided primary antibodies Bcl-2–associated X protein (Bax) (1:500) and heat shock protein (Hsp-70) (1:100) (Santa Cruz Biotechnology Inc, California, USA) for 15 minutes. Following re-washing, Streptavidin-HRP added and the sections left in incubator ~15 min than washing. The chromagen diaminobenzidine-substrate added to the sections ~10 min incubation followed by washing and hematoxylin counterstaining for five seconds. Weak ammonia (0.037 mol/L) was used in dipping the tissue sections which are finally washed and cover slipped to be examined using light microscopy. Positive antigens were brown stained under the microscope.

3.9.7. Oxidative Damage Evaluation from the Gastric Tissue Homogenate

3.9.7.1. Sample preparation

For assaying PGE_2, SOD and MDA in the gastric tissue of all ainmal groups, small pieces of gastric tissues were and weighed (about 200 mg for each) (Yildirim et al., 2007). The tissues were homogenized in Teflon homogenizer (Polytron, Heidolph RZR 1, Germany) was used to homogenize the tissues using phophate buffer saline. After centrifugation at 18,000 x g for 15 min at 4°C, the supernatant was collected and divided into aliquots.

3.9.7.2. Measurement of SOD Activity

The homogenized stomach tissues were left in room temperature prior to perform this assay. The activity of SOD was measured using the protocol provided by Cayman's SOD Assay Kit, Item Number 706002, USA. The details are explained in Appendix II.

3.9.7.3. Measurement of Membrane Lipids Peroxidation (MDA)

The rate of lipoperoxidation in the gastric mucous membrane was estimated by determination of malondialdehyde (MDA) using the Thiobarbituric Acid Reactive Substances (TBARS) test. (Catalogue no.10009055).Details in Appendix II.

3.9.7.4. Measurement of PGE2 enzyme level

The level of PGE2 enzyme levels in gastric mucosa was evaluated following the procedure described in Prostaglandin E2 EIA Kit-Monoclonal Cayman Chemical (Item Number 514010), USA. The details are explained in Appendix II.

3.7. Statistical Analysis

Data obtained were presented in form of mean ± SEM, and the significance within the groups was analysed using one-way ANOVA and tukey analysis, $P<0.05$ was considered significant.

CHAPTER IV

RESULTS

4.1. Plant Extraction

Air-dried powders of the leaves of each plant were extracted by using ethanol solvents. On the other hand, ethanol was selected as a polar solvent that is highly volatile solvent has higher penetration susceptibility. Solvents with different polar have been used for plant extraction and researchers predicted that ethanol is capable to obtain a higher quantity of crude extract compared to water (Pinelo *et al.*, 2005). Many researchers have emphasized that the type of solvent affects on the extraction yield (Kumar *et al.*, 2008; Ling *et al.*, 2010). This is in accordance with the present findings, which show that all the plants promote high extract yield with ethanol extraction solvent. As exhibited by using the ethanolic solvent the amount of 9.75 and 11.3 g/100 g of dried leaf plant have been yielded from *Jasminum Sambac and Hemigraphis Colorata* respectively. It can be explained that these plants contain compounds that can be better dissolved in ethanolic solvent. Previous studies have shown same results for other plant extracts such as grape pomace (Pinelo *et al.*, 2005). Herrrero *et al.*, (2005) was deduced that ethanol extracts provides the highest extraction yields and a good efficiency of the extraction process in comparison with other polarity solvents including water.

4.2. Acute Toxicity Study

All experimental animals participated in acute toxicity study were healthy,active and didn’t manifest any significantly sign of toxicity even at high dose (5g/kg),(Figure 4.1, 4.2 and Table 4.1, 4.2).

Table 4.1: (A, B, C, D).

A- Effect s of *J. sambac* leaf extract on kidney biochemical parameters in male rats.

Dose	Sodium (mmol/L)	Pottasium (mmol/L)	Chloride (mmol/L)	CO_2 (mmol/L)	Anion gap (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Vehicle (CMC)	141.33 ± 0.58	4.98 ± 0.04	103.88 ± 0.82	24.12 ± 0.54	18.75 ± 0.46	5.45 ± 0.45	34.83 ± 2.17
LD (2 g/kg)	142.05 ± 0.55	5.02 ± 0.09	105.29 ± 1.02	22.67 ± 0.81	18.55 ± 0.62	6.06 ± 0.83	33.87 ± 2.29
HD (5 g/kg)	143.14 ± 0.68	4.91 ± 0.06	104.35 ± 0.54	23.90 ± 0.64	19.15 ± 0.45	5.63 ± 0.37	35.05 ± 2.26

B- Effect s of *J. sambac* extract on kidney biochemical parameters in female rats.

Dose	Sodium (mmol/L)	Pottasium (mmol/L)	Chloride (mmol/L)	CO_2 (mmol/L)	Anion gap (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Vehicle (CMC)	141.87 ± 0.42	4.83 ± 0.14	105.78 ± 0.67	23.33 ± 0.41	18.00 ± 0.25	7.95 ± 0.33	41.76 ± 2.75
LD (2 g/kg)	142.07 ± 0.56	4.55 ± 0.16	105.85 ± 0.65	22.65 ± 0.42	17.49 ± 0.46	7.97 ± 0.49	42.00 ± 2.36
HD (5 g/kg)	142.15 ± 0.47	4.63 ± 0.18	107.03 ± 0.53	21.96 ± 0.75	17.67 ± 0.48	8.31 ± 0.68	43.13 ± 2.24

Values expressed as mean ± S.E.M. There are no significant differences between groups. Significant value at $P<0.05$

C- Effect s of *J. sambac* extract on liver biochemical parameters in male rats.

Dose	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	TB (µmol/L)	CB (µmol/L)	AP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Vehicle (CMC)	60.45 ± 1.25	9.62 ± 0.49	52.02 ± 1.40	2.17 ± 0.17	1.00 ± 0.00	153.00 ± 6.35	50.08 ± 1.62	172.95 ± 6.13	3.26 ± 0.25
LD (2 g/kg)	58.86 ± 0.86	8.81 ± 0.38	50.71 ± 1.21	2.13± 0.16	1.00 ± 0.00	154.17 ± 8.10	48.33 ± 0.58	174.23 ± 5.14	3.65 ± 0.42
HD (5 g/kg)	60.15 ± 1.05	9.17 ± 0.46	50.33 ± 1.24	2.02 ± 0.13	1.00 ± 0.00	155.00 ± 7.04	47.87 ± 1.55	175.15 ± 7.02	3.37 ± 0.18

D- Effect s of *J. sambac* extract on liver biochemical parameters in female rats.

Dose	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	TB (µmol/L)	CB (µmol/L)	AP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Vehicle (CMC)	64.33 ± 1.26	11.19 ± 0.17	53.17 ± 1.28	2.00± 0.00	1.00 ± 0.00	108.83 ± 4.13	43.17 ± 2.91	171.83 ± 6.38	3.67 ± 0.33
LD (2 g/kg)	63.75 ± 1.19	11.05 ± 0.45	52.33 ± 1.26	2.00 ± 0.00	1.00 ± 0.00	98.83 ± 5.25	42.96 ± 2.70	172.17 ± 6.35	3.50 ± 0.51
HD (5 g/kg)	65.02 ± 2.65	11.30 ± 0.43	53.02 ± 1.25	2.00 ± 0.00	1.00 ± 0.00	102.67 ± 5.17	44.02 ± 1.85	174.28 ± 5.26	3.22 ± 0.44

TB: Total bilirubin; CB: Conjugated bilirubin; AP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase;GGT: G-Glutamyl Transferase. Data are presented in the form of mean ± S.E.M. No significant differences among groups, Significant value at $P<0.05$.

Table 4.2: (A, B, C, D). Acute toxicity test results of *H. colorata*

A- Effect of *H. colorata* on renal function test of male rats.

Dose	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	CO_2 (mmol/L)	Anion gap (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Vehicle (CMC)	144.12 ± 0.81	4.69 ± 0.05	105.37 ± 2.17	24.44 ± 0.80	19.24 ± 0.78	5.83 ± 0.55	33.65 ± 1.66
LD (2 g/kg)	142.33 ± 0.67	5.04 ± 0.08	106.52 ± 1.99	24.27 ± 0.86	17.83 ± 0.82	6.14 ± 0.74	33.95 ± 1.93
HD (5 g/kg)	145.17 ± 0.87	5.11 ± 0.07	107.07 ± 2.04	23.82 ± 0.91	19.15 ± 0.69	6.02 ± 0.34	35.37 ± 2.52

B- Effect of *H. colorata* on renal function test of female rats.

Dose	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	CO_2 (mmol/L)	Anion gap (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Vehicle (CMC)	143.29 ± 1.26	4.81 ± 0.23	105.55 ± 1.37	23.18 ± 0.54	18.39 ± 0.37	7.69 ± 0.35	42.17 ± 1.70
LD (2 g/kg)	142.35 ± 1.56	4.48 ± 0.27	106.63 ± 1.58	20.65 ± 0.62	17.54 ± 0.48	8.37 ± 0.28	44.32 ± 1.21
HD (5 g/kg)	144.48 ± 1.38	5.03 ± 0.18	107.18 ± 2.17	21.25 ± 0.71	19.27 ± 0.37	8.56 ± 0.65	43.09 ± 1.33

Data are presented in the form of mean ± S.E.M. No significant differences between groups. Significant value at $P<0.05$

C- Effect of *H. colorata* on liver function test of male rats.

Dose	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	TB (µmol/L)	CB (µmol/L)	AP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Vehicle (CMC)	62.14 ± 1.56	9.76 ± 0.58	52.27 ± 1.33	2.21 ± 0.18	1.00 ± 0.00	154.35 ± 4.13	51.26 ± 1.88	176.65 ± 6.52	3.22 ± 0.17
LD (2 g/kg)	59.89 ± 2.06	10.11 ± 0.48	50.83 ± 1.53	2.08 ± 0.15	1.00 ± 0.00	156.61 ± 5.42	48.38 ± 1.78	180.14 ± 5.43	3.76 ± 0.33
HD (5 g/kg)	61.55 ± 1.82	9.35 ± 0.54	51.50 ± 1.25	2.01 ± 0.17	1.00 ± 0.00	157.08 ± 5.54	49.10 ± 1.89	178.33 ± 4.67	3.83 ± 0.257

D- Effect of *H. colorata* on liver function test of female rats.

Dose	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	TB (µmol/L)	CB (µmol/L)	AP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Vehicle (CMC)	65.50 ± 1.45	11.34 ± 0.25	53.65 ± 1.25	2.00 ± 0.00	1.00 ± 0.00	107.55 ± 5.05	43.70 ± 1.97	171.68 ± 5.80	3.60 ± 0.32
LD (2 g/kg)	63.27 ± 1.35	12.05 ± 0.40	52.06 ± 1.27	2.00 ± 0.00	1.00 ± 0.00	102.37 ± 5.47	45.56 ± 1.85	174.33 ± 6.54	3.54 ± 0.52
HD (5 g/kg)	64.67 ± 1.88	11.56 ± 0.34	50.78 ± 0.54	2.00 ± 0.00	1.00 ± 0.00	104.17 ± 5.26	44.29 ± 1.67	176.86 ± 5.83	3.09 ± 0.45

TB: Total bilirubin; CB: Conjugated bilirubin; AP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase;GGT: G-Glutamyl Transferase. Data are presented in the form of mean ± S.E.M. No significant differences between groups, Significant value at $P<0.05$.

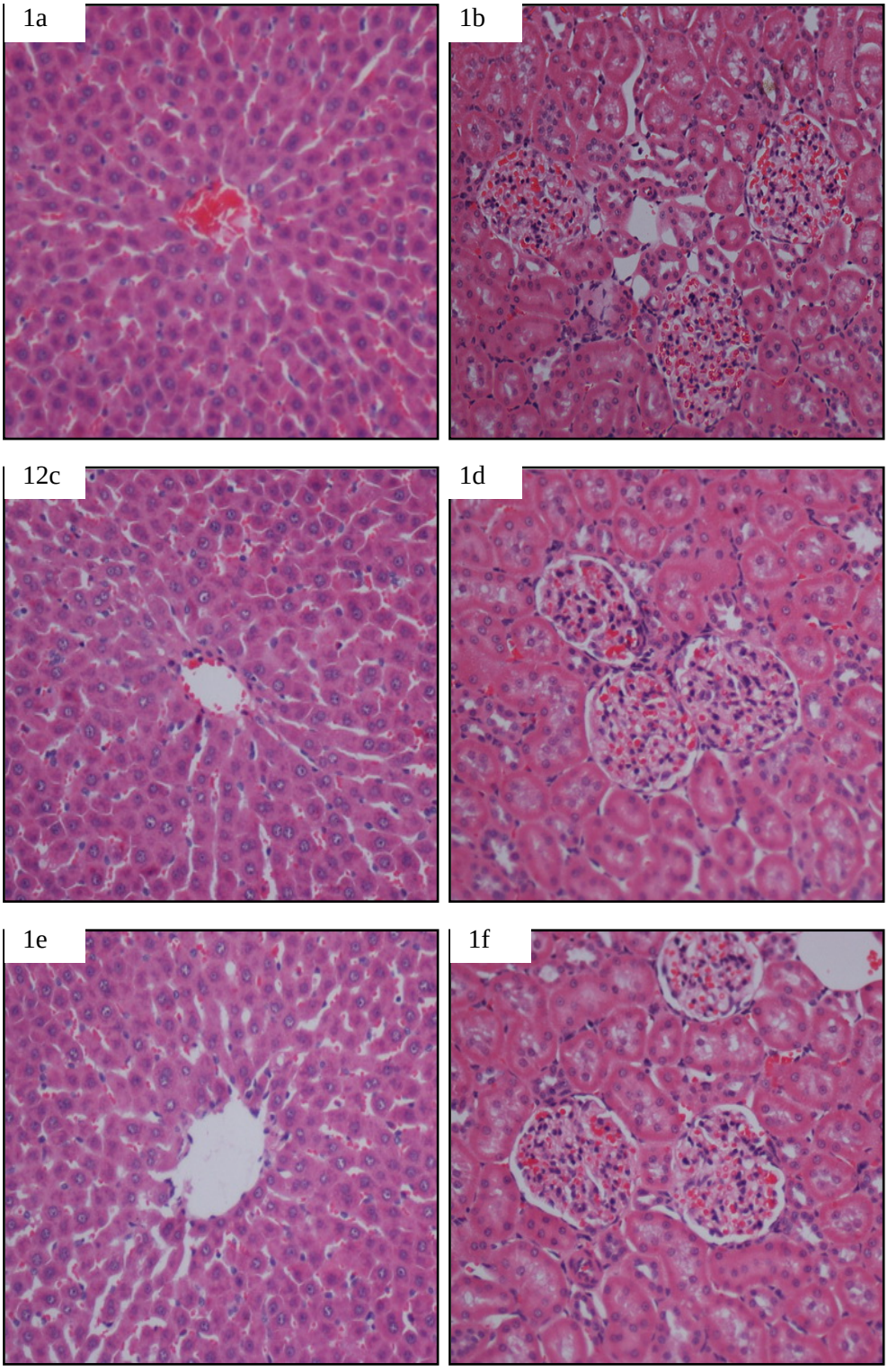


Figure4.1: Histological sections of the liver and kidney from the acute toxicity test**.** (1a and 1b) Rats treated with 5 mL/kg of the vehicle (CMC). (1c and 1d) Rats treated with 2 g/kg (5 mL/kg) of the *J. sambac* extract. (1e and 1f) Rats treated with 5 g/kg (5 mL/kg) of the *J. sambac* extract. There is no significant difference in the structures of the livers and kidneys between the treated and control groups (Hematoxylin and Eosin stain, 20 x magnifications).

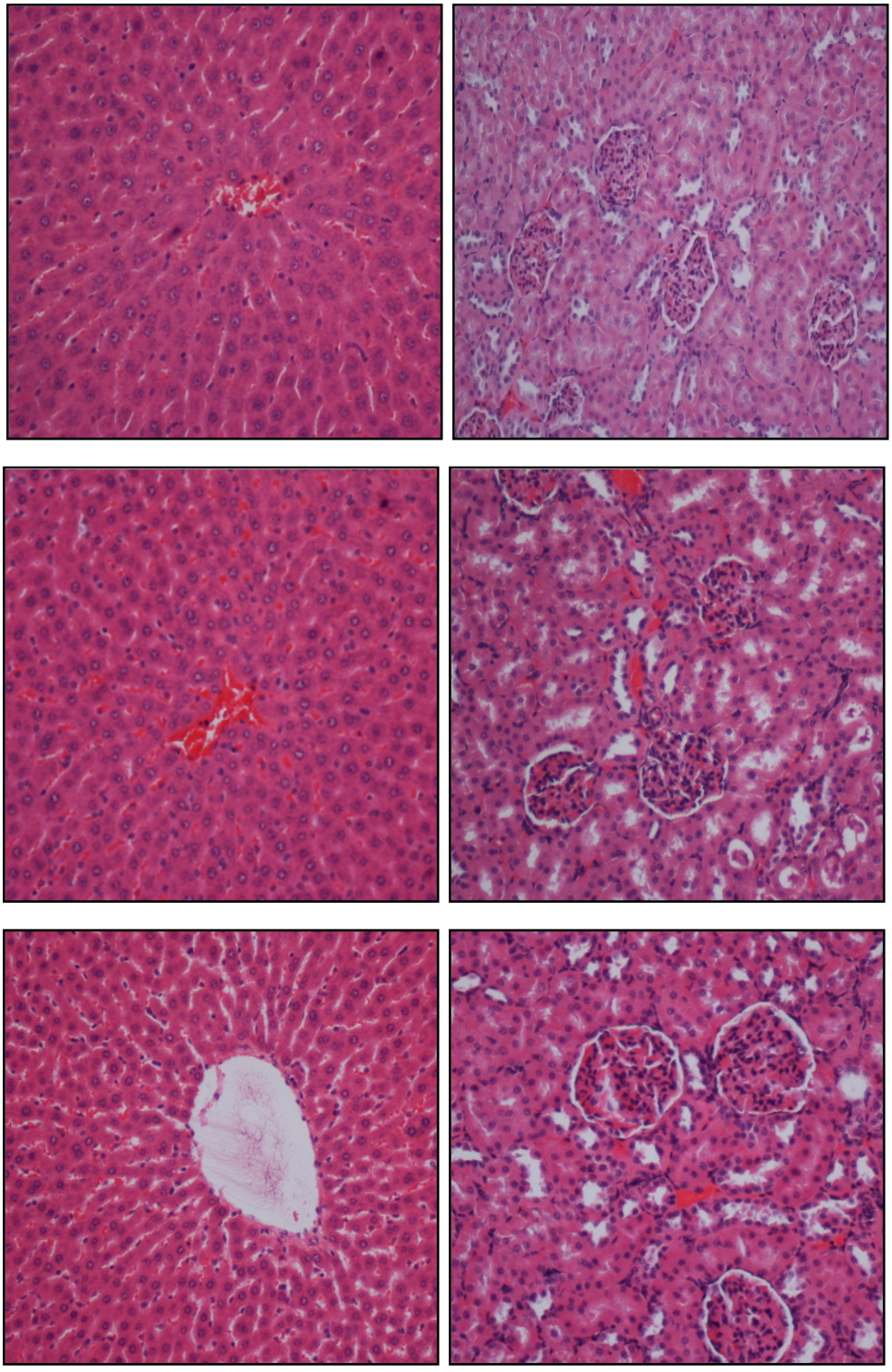


Figure 4.2: Histological sections of the liver and kidney from the acute toxicity test**.** (1a and 1b) Rats treated with 5 mL/kg of the vehicle (CMC). (1c and 1d) Rats treated with 2 g/kg (5 mL/kg) of the *H. colorata* extract. (1e and 1f) Rats treated with 5/kg (5 mL/kg) of the *H. colorata* extract. There is no significant difference in the structures of the livers and kidneys between the treated and control groups (Hematoxylin and Eosin stain, 20 x magnifications).

4.3. Antioxidant Properties of *J. sambac* (JS) and *H. colorata* (HC) Extracts

Generally, antioxidant substances in fruit and vegetable play an important role as a health protecting aspect. Scientific research proves that antioxidants are able to decrease the risk of chronic diseases including cancer, heart disease and peptic ulcer. The primary sources of plant antioxidants are vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens that have been established as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties (Velioglu *et al.*, 1998).

Different antioxidant activity assays have been utilized to detect the antioxidant activity of foods. In this study, DPPH radical scavenging activity assay and total phenolic content have been performed to monitor the antioxidant activity of *J. sambac* and *H. colorata* Crude extracts. DPPH assay showed that the concentration of *J. sambac* crude exract need to scavenge 50 % of DPPH is 23.33 ± 0.33 µg/mL and approaching the value BHT (22.47 ± 0.77 µg/mL). On the other hand, *H. colorata* crude extract showed higher IC50 > 50 µg/mL indicating that the DPPH scavenging power of *J.sambac* extract is higher than that of *H. colorata* extract. The total phenolic content of *J. sambac* crude extract showed higher value (65.70 ± 0.25 mg GA/mg extract) compared to less value in *H. colorata* (45.70 ± 0.22 mg GA/mg extract). Figure **4.3** illustrates the Total phenolic content of *J. sambac* and *H. colorata* Crude extracts and the results recorded higher value of JS (65.70 ± 0.25 mgGAE/mg extract compared to HC which recorded 45.64 ± 0.22 mg GAE/mg extract. The results indicate that the total phenolic constituent of JS is better than that of HC.

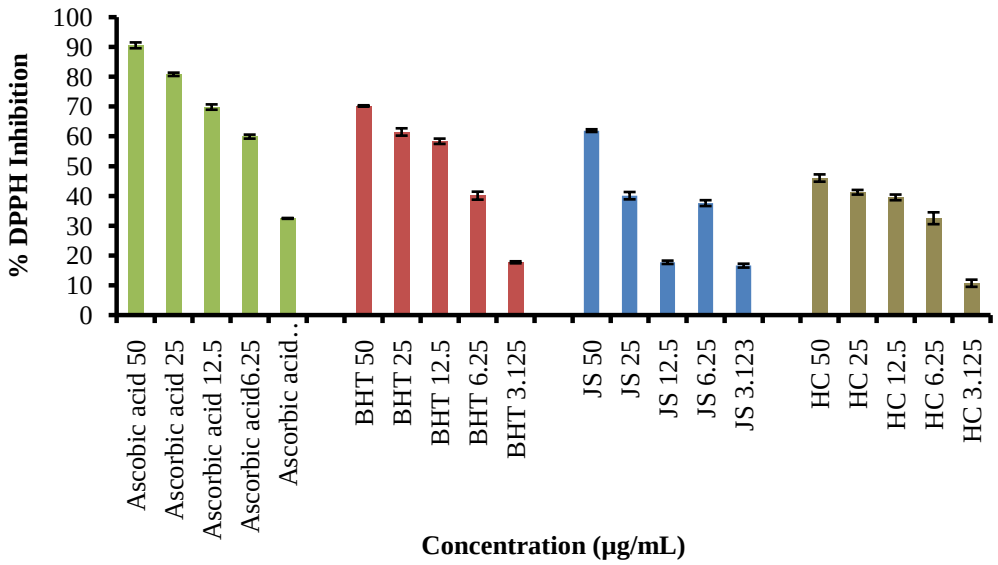


Figure4. 3: Inhibition of DPPH activity of *J.sambac* and *H. colorata* leaf extracts compared to the standards Ascorbic acid, and Trolox.

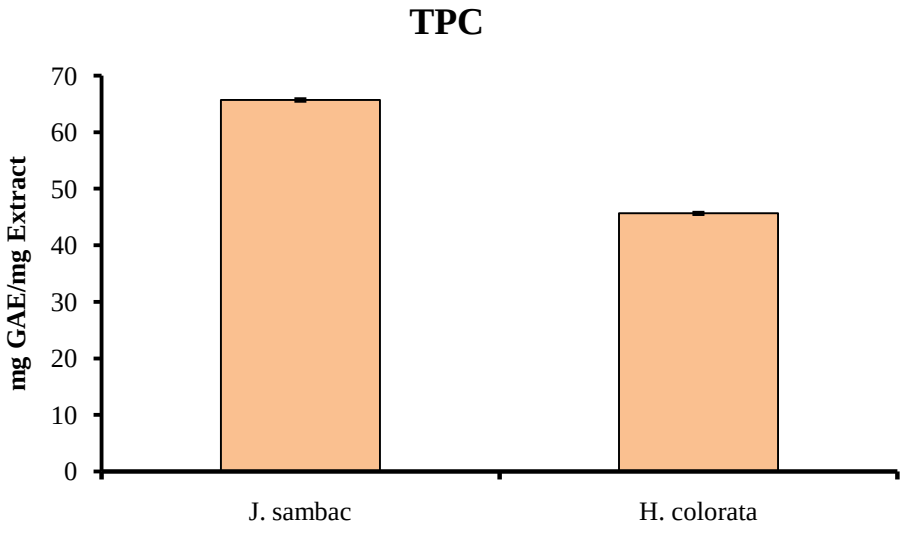


Figure 4.4: Total phenolic content (TPC) of *J.sambac* and *H. colorata* leaf extracts in gallic acid equivalent

4.4. Wound healing experiment

4.4.1. Wound Closure Area

The rate of wound healing activity was evaluated by a blind observer unaware of the experimental protocol. Macroscopically, wounds dressed with *J. sambac* and *H. colorata* showed considerable signs of dermal healing and significantly healed faster compared to the placebo-treated control group (gum acacia in normal saline) (Figures 5a,b and 7a,b). In addition, Dressing of wounds with *J. sambac* and *H. colorata* resulted in smaller wounds and had significantly less % wound closure areas on Day 10 post-wounding (20.67 ± 1.86 and 19.39 ± 1.53% respectively for low dose and 10.53 ± 1.52 and 9.53 ± 1.05% respectively for high dose of each plant extract) compared with intrasite gel, the reference control (17.50 ± 1.15 and 17.17 ± 1.11% respectively) and the placebo (30.35 ± 2.70 and 28.55 ± 1.20%) used for each plant extract respectively (Figures 4.6 and 4.8). These results indicate the efficacy of both plant extracts in accelerating wound healing.

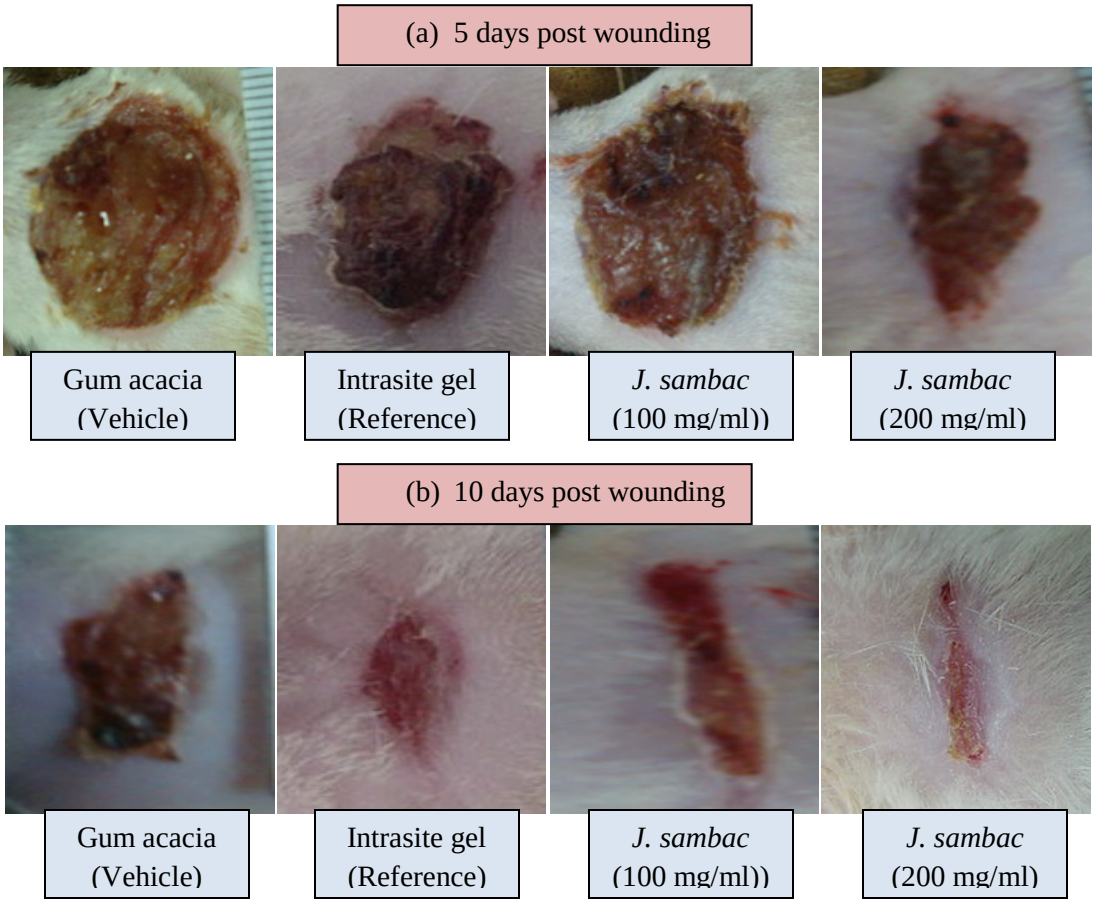


Figure 4.5: Effect of *J. sambac* ethanol extract on the macroscopical appearance of excision injury healing area treatment at Day 5 of post-injury (a), and at Day day10 of post-injury

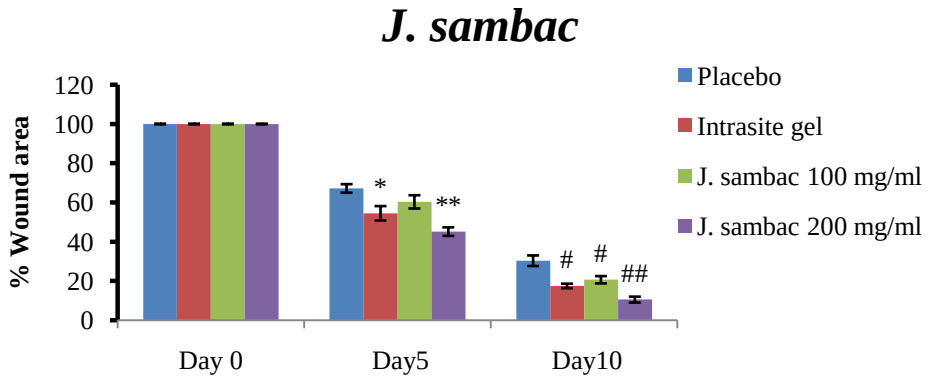


Figure 4.6: Effect of *J. sambac* ethanol extract on the wound area % in day 0, 5th and 10th post wounding of placebo control, Intrasite gel, *J. sambac* (100 mg/ml) and *J. sambac* (200 mg/ml) in rats. All values expressed as Mean ± SEM. Mean with different superscript was significantly difference. The mean difference is significant at the 0.05 level.

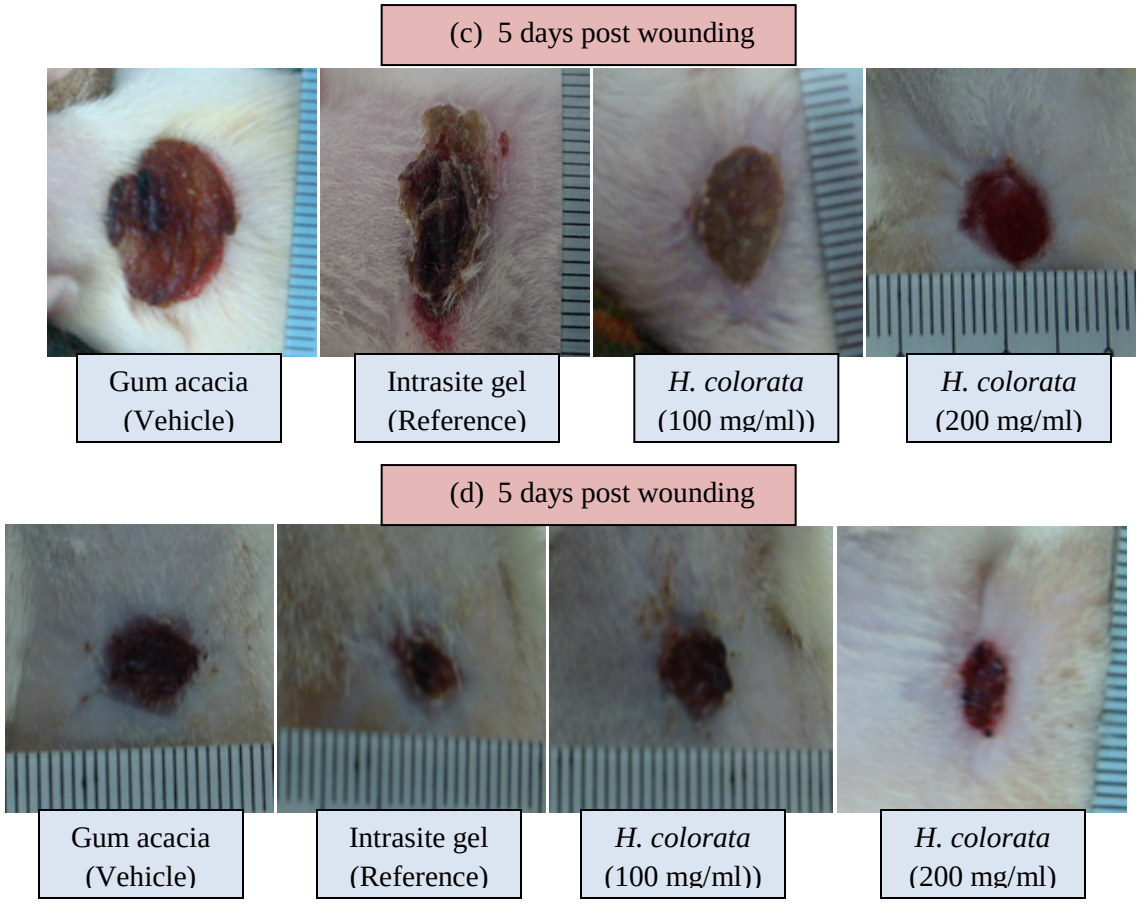


Figure4.7: Effect of *H. colorata* ethanol extract on the macroscopical appearance of excision injury healing area treatment at Day 5 of post-injury (a), and at Day day10 of post-injury

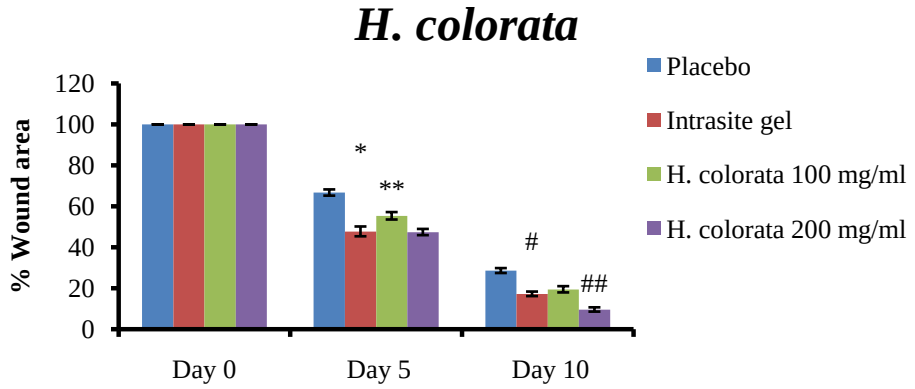


Figure 4.8: Effect of *H. colorata* ethanol extract on the wound area % in day 0, 5th and 10th post wounding of placebo control, Intrasite gel, *H. colorata* (100 mg/ml) and *H. colorata* (200 mg/ml) in rats. All values expressed as Mean ± SEM. Mean with different superscript was significantly difference. The mean difference is significant at the 0.05 level.

4.5. Histology of wound tissues

Haematoxylin and Eosin, and Masson trichrome stains

The histology of wound tissues on day 10 post-wounding of samples collected from all animal groups showed that the wound enclosure in the tissues treated with *J.* sambac and *H. colorata* ethanol extracts were smaller and the granulation tissues contained comparatively few inflammatory cells, and greater collagen, fibroblast and proliferating blood capillaries compared with placebo-treated control group (Figures 4.8 and 4.9). The higher collagen content of wounds as assessed by the H & E staining was better brought out on staining with Masson's trichrome (Figure 4.10 and 4.11). In addition, the alignment of the collagen fibers was also much better in *J. sambac* and *H. colorata*-treated wounds in that they were compact and parallel to the surface indicating the stimulation and enhancement of the lay down of collagen fibers and new blood vessels in granulation tissue than placebo-treated control group.

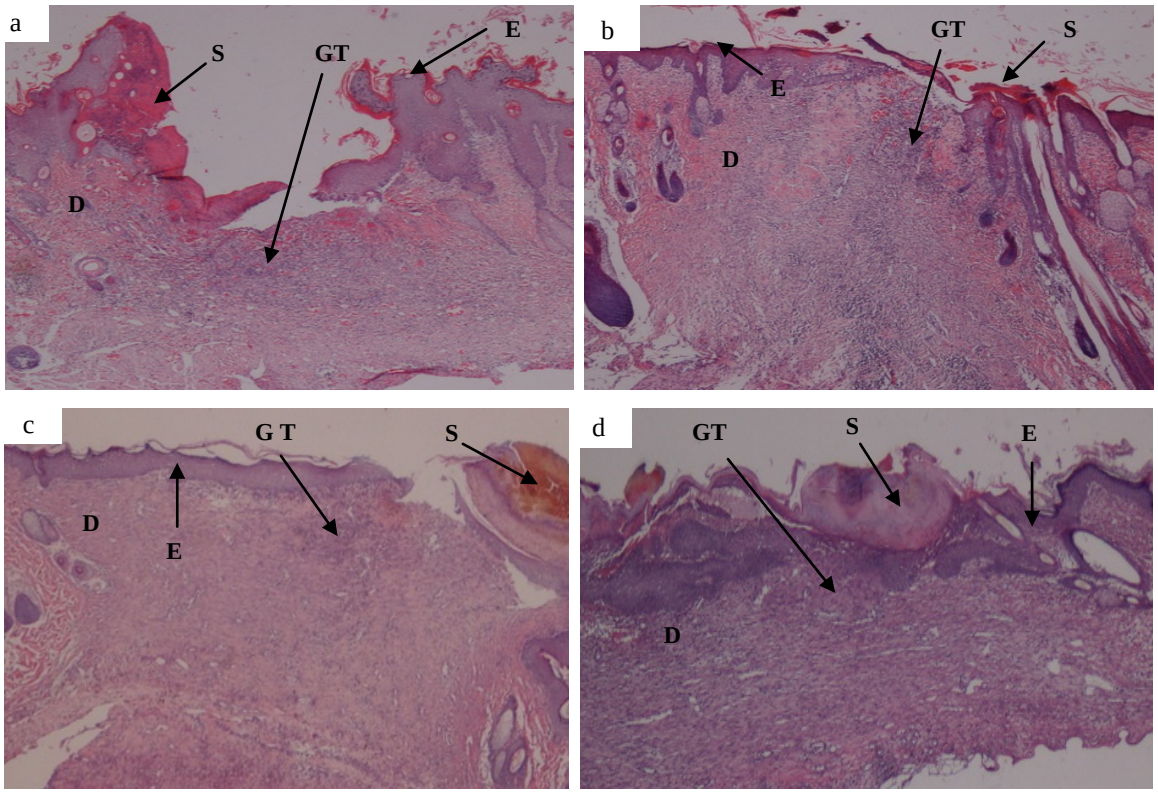


Figure 4.9: Hematoxylin and eosin-stained sections of the wound treated with *J. sambac* ethanol extract at Day 10 of post-wounding in rats. (a) 0.2 ml of placebo, gum acacia in normal saline. (b) 0.2 ml Intrasite gel. (c) 0.2 ml *J.Sambac (*100 mg/kg). (d) 0.2 ml *J.Sambac* (200 mg/kg). S = Scab, E = Epidermis, GT = granulation tissue (magnification 20x).

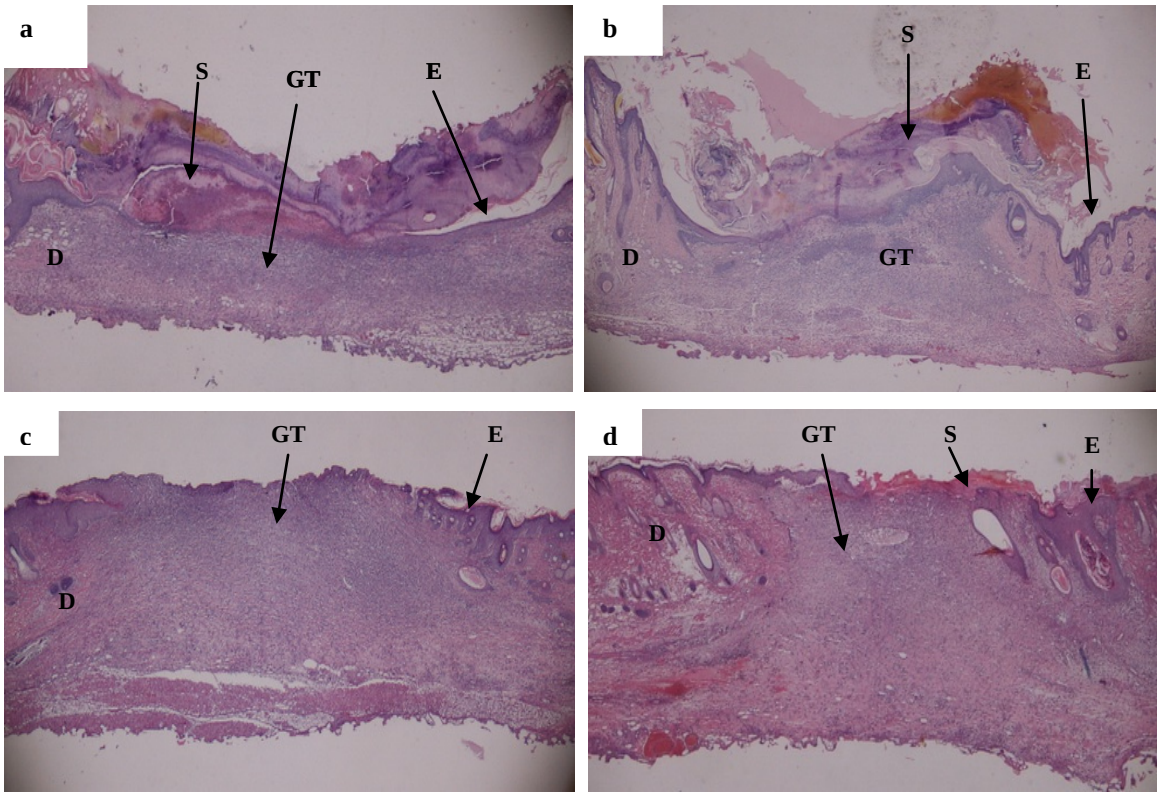


Figure4.10: Hematoxylin and eosin-stained sections of the wound at Day 10 of post-wounding in rats treated with *H. colorata* ethanol extract. (a) 0.2 ml of placebo, gum acacia in normal saline. (b) 0.2 ml of Intrasite gel. (c) 0.2 ml of *H. colorata (*100 mg/kg). (d) 0.2 ml of *H. colorata* (200 mg/kg). S = Scab, E = Epidermis, GT = granulation tissue (magnification 20x).

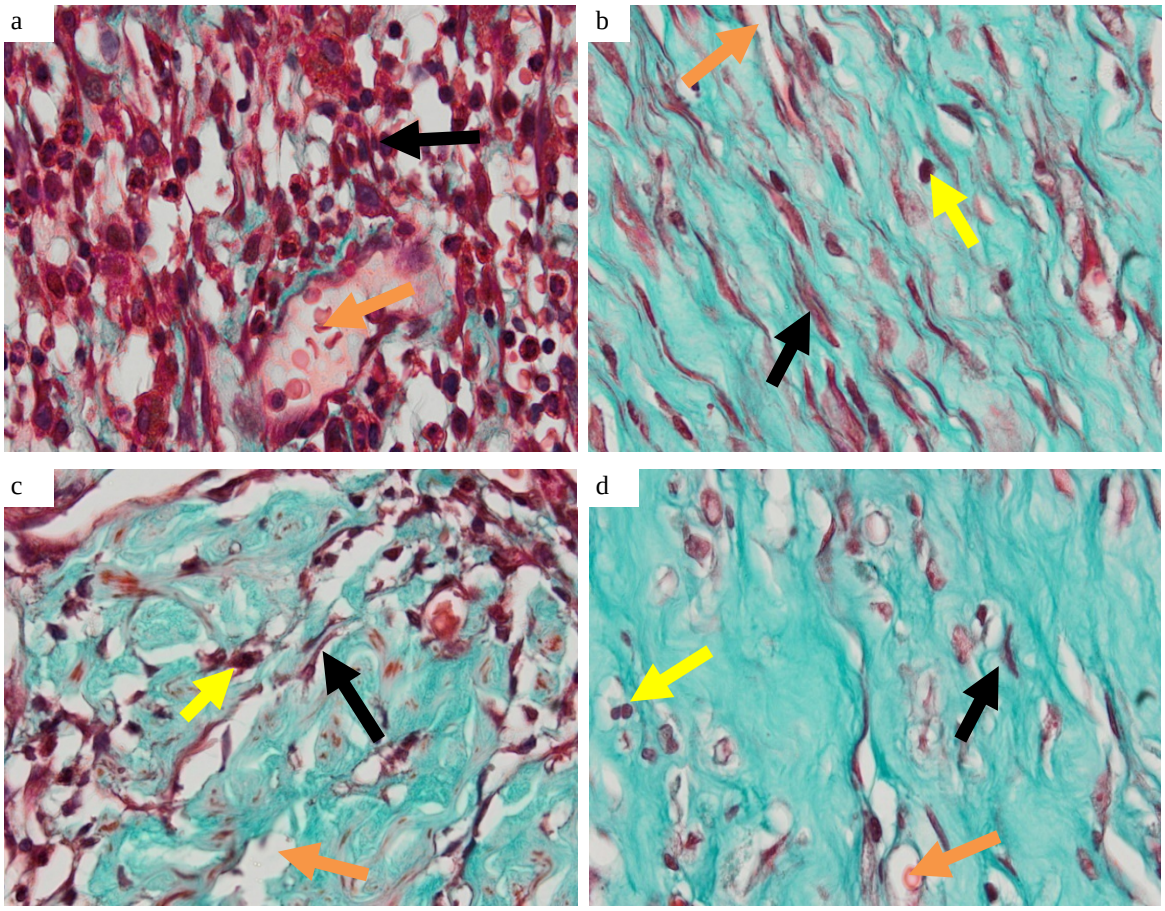


Figure 4.11: Masson's Trichrome-stained sections of the granulation tissue in wounds at Day 10 post-wounding in rats treated with *J. sambac* ethanol extract (a) Wound dressed with gum acacia shown granulation tissue harbors comparatively more inflammation cells (mononuclear cells) (black arrow) than the granulation tissue shown in (b, c, and d) (yellow arrow). Wound dressed with Intrasite gel or with *J. sambac* (100 and 200 mg/kg) shows comparatively more deposition of collagen (green color) in the area of the wound and increase in the fibroblast (black arrow) and angiogenesis (orange arrow) relative to the granulation tissue shown in (a) (magnification 100x).

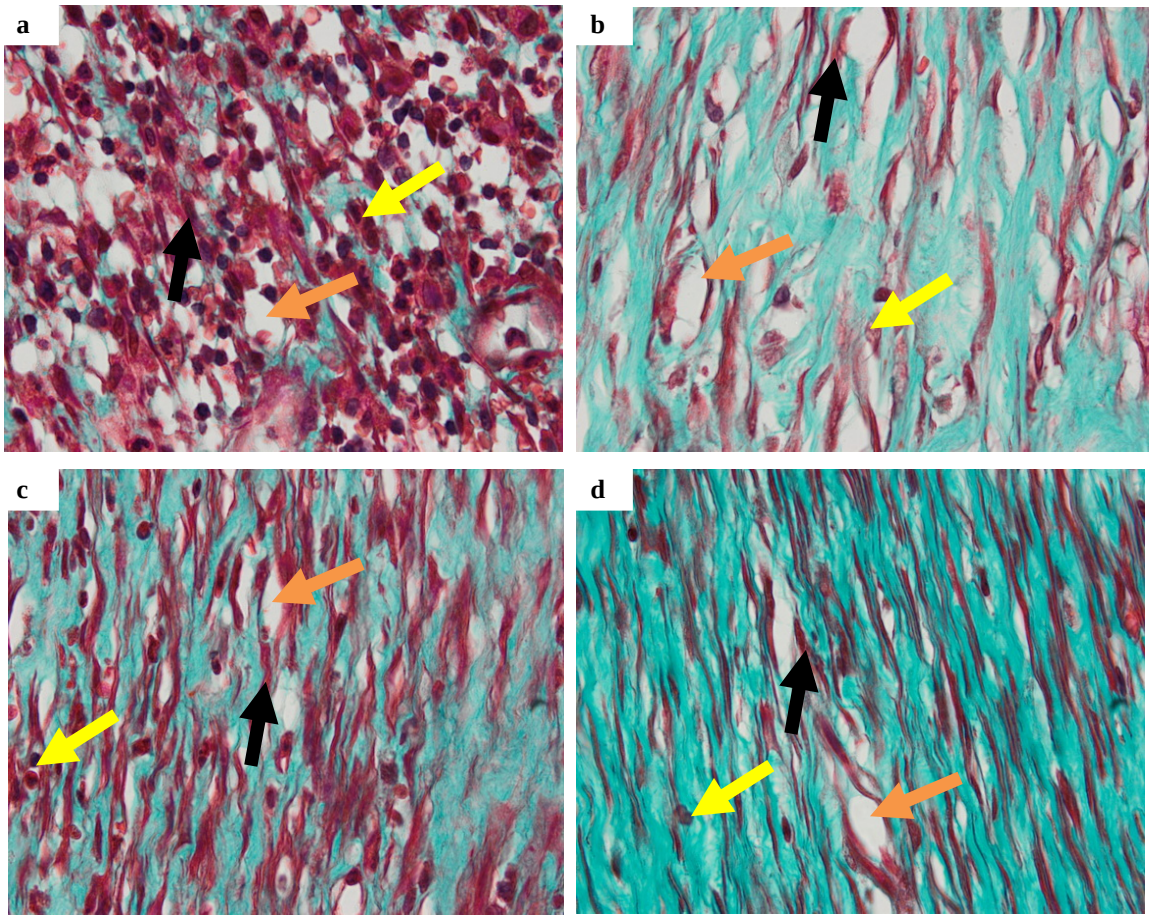


Figure 4.12: Masson's Trichrome-stained sections of the granulation tissue in wounds at Day 10 post-wounding in rats.treated with *H. colorata* ethanol extract (a) Wound dressed with gum acacia shown granulation tissue harbors comparatively more mononuclear cells (black arrow) than the granulation tissue shown in (b, c, and d) (yellow arrow). Wound dressed with Intrasite gel or with *H. colorata* (100 and 200 mg/kg) shows comparatively more deposition of collagen (green color) in the area of the wound and increase in fibroblast (black arrow) and angiogenesis (orange arrow) relative to the granulation tissue shown in (a) (magnification 100x).

4.6. Total protein content

Figures 4.13 and 4.14 showed the total protein in the wound tissue area taken from each animal on Day 10 post-wounding. The total protein content was significantly higher in the tissue homogenate from *J. sambac* and *H. colorata*-treated animals and Intrasite gel than the placebo-treated group. Whereas, higher protein levels were significantly detected in the rats dressed with the high dose of *J. sambac* extract (200 mg/ml) (4.00 ± 013 mg/100 g wet weight), but the low dose (100 mg/ml) did not show any significant increase in the protein content level (2.30 ± 0.29 mg/100 g wet weight) compared with the placebo (1.97 ± 0.24 mg/100 g weight) (Figure 12). However, the doses of *H. colorata* (200 mg/ml and 100 mg/ml respectively) exhibited significant increase in the level of protein (4.33 ± 0.14 and 3.81 ± 0.20 mg/100 g wet weight) compared with that of its placebo (1.79 ± 0.04 mg/100 g wet weight). These results indicate that both plant extract can improve the level of the protein content in the wound area depending on the dose given to the animal.

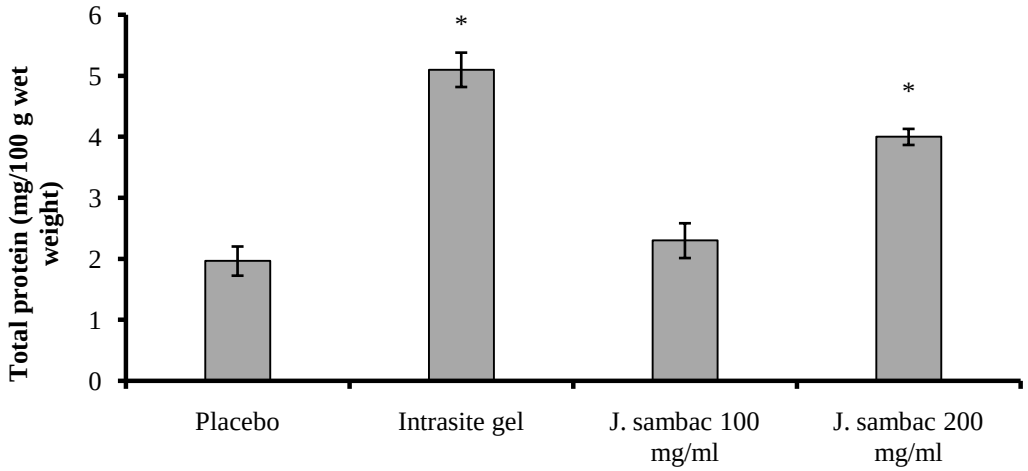


Figure 4.13: Effect of *J.* sambac ethanol extract on the total protein from the granulation tissue in Day 10 post wounding of placebo control, Intrasite gel, *J. sambac* (100 mg/ml) and *J. sambac* (200 mg/ml) in rats. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to placebo

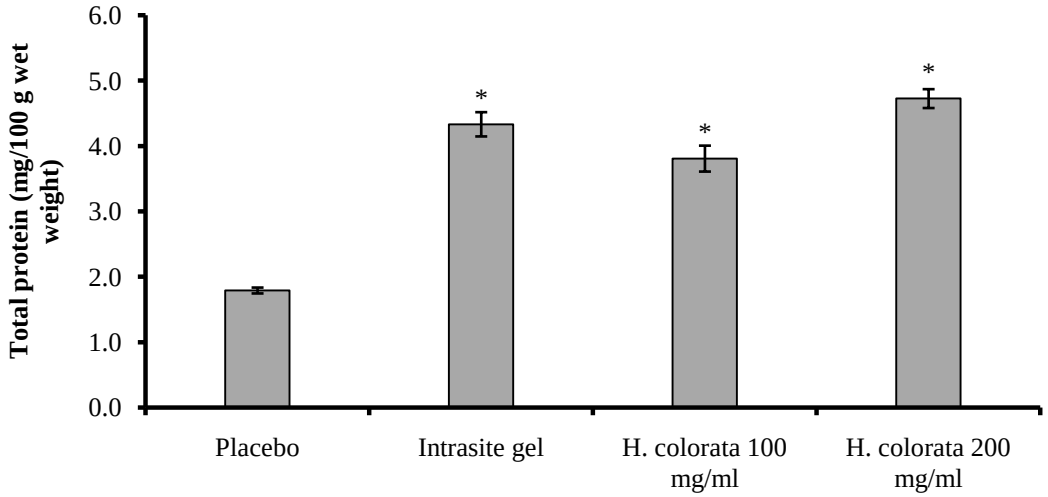


Figure 4.14: Effect of *H. colorata* ethanol extract on the total protein from the granulation tissue in Day 10 post wounding of placebo control, Intrasite gel, *H. colorata* (100 mg/ml) and *H. colorata* (200 mg/ml) in rats. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to placebo

4.7.Antioxidant enzymes and MDA levels in wound tissues

The antioxidant enzymes GPx and SOD activities and MDA levels were calculated, taking into account the amount of protein present in the healing wound, as this indicates the number of living cells present. Results of *J. sambac* treatment showed that GPx and SOD antioxidant enzymes were significantly elevated (*P*<0.05) in rats receiving high dose *J. sambac* extract (200 mg/ml) (35.03 ± 3.55 and 39.31 ± 2.78 U/mg protein respectively compared with placebo-treated control group (14.12 ± 0.70 and 18.57 ± 1.06 U/mg protein respectively). Treating the wounds of animals with low dose *J. sambac* extract didn't show significant increase in GPx and SOD (20.48 ± 1.98 and 26.31 ± 1.99 U/mg protein respectively) compared with the placebo control and the intrasite gel-treated groups (27.78 ± 1.63 and 32.48 ± 2.90 U/mg protein respectively. (Figure 4.15). On the other hand there was significant reduction in MDA levels observed in tissue taken from rats treated with low and high dose of *J.sambac* extract (51.00 ± 8.02 and 18.04 ± 3.91 nmol/mg protein

respectively compared with placebo-treated control group (100.50 ± 8.34 nmol/mg protein (Figure 4.16). As for *H. colorata* extract, both low dose and high dose showed significant increase in the antioxidant enzymes GPx and SOD in the tissue homogenate collected from all animal groups (22.47 ± 2.12 and 29.17 ± 1.99 U/mg protein respectively for the low dose-treated group and 32.03 ± 1.83 and 35.71 ± 2.44 U/mg protein respectively for the high dose-treated groups) compared with the placebo group (15.37 ± 0.47 and 20.55 ± 0.60 U/mg protein respectively. On the contrary, the level of MDA was significantly reduced in both doses of *H. colorata*-treated groups (24.39 ± 2.44 and 11.50 ± 0.66 nmol/mg protein respectively) compared with the placebo group (90.74 ± 4.24 nmol/mg protein). These results suggest that *J.sambac* and *H. colorata* are both effective in wound healing.

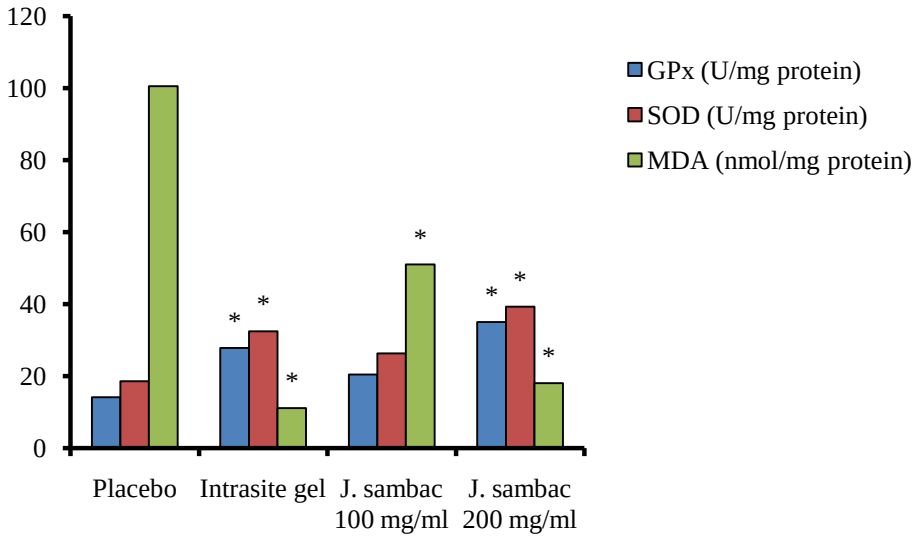


Figure 4.15: Effect of *J. sambac* ethanol extract on GPx, SOD and MDA levels in wound tissue homogenates on day 10 post-wounding of placebo control, Intrasite gel, *J.sambac* (100 mg/ml) and *J. sambac* (200 mg/ml) treated rats. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to placebo.

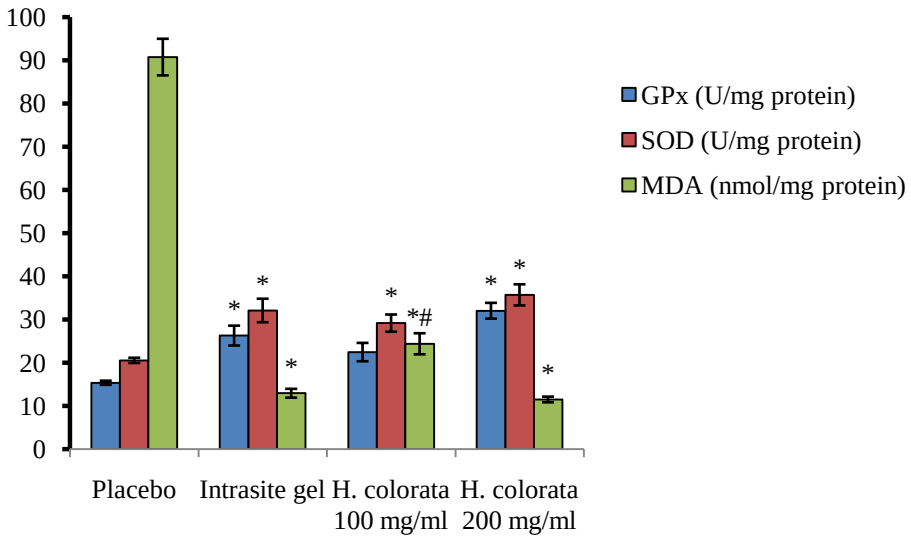


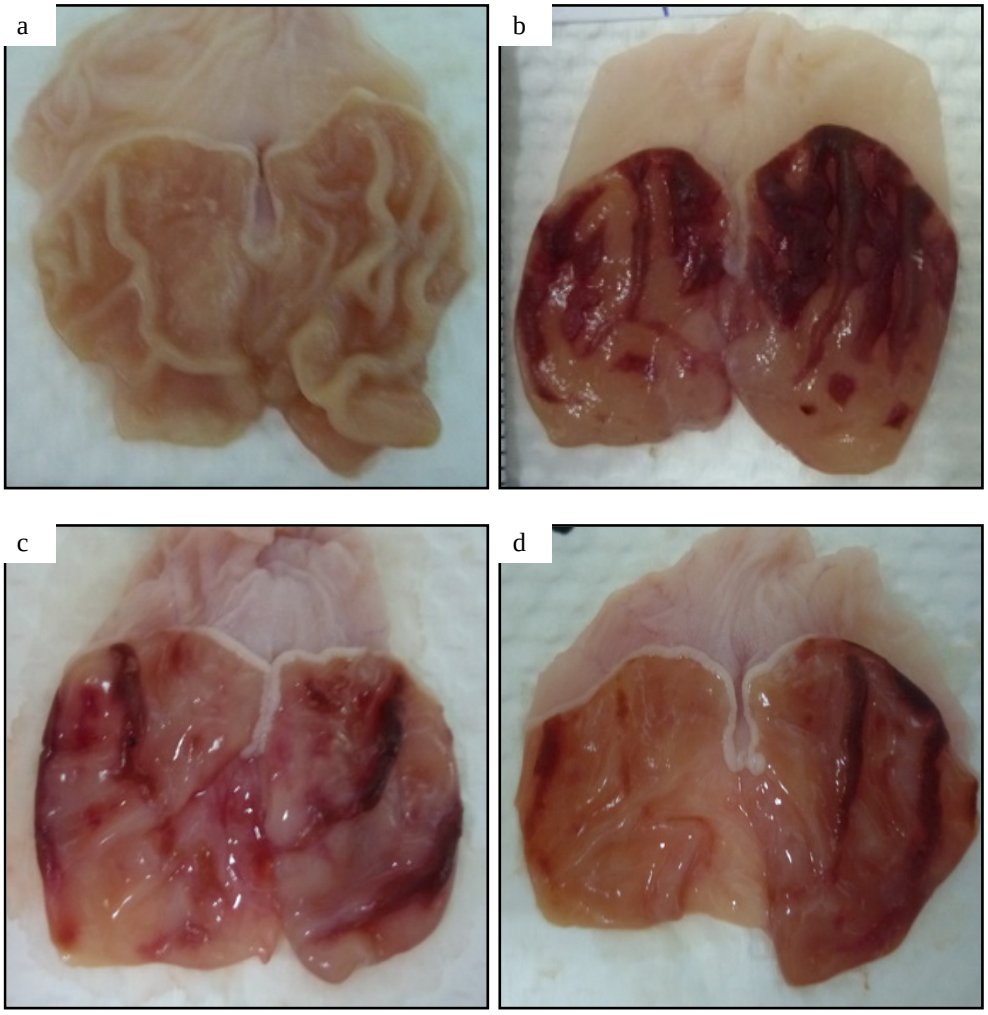
Figure 4.16: Effect of *H. colorata* ethanol extract on GPx, SOD and MDA levels in wound tissue homogenates on day 10 post-wounding of placebo control, Intrasite gel, *H. colorata* (100 mg/ml) and *H. colorata* (200 mg/ml) treated rats. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to placebo. #*P*<0.01 compared with intrasite gel-treated group.

4.8. Gastroprotective Activity of *J. sambac* and *H. colorata* Leaf Extract and their isolated Active Compounds β-sitosterol and Octyl gallate against HCl/Ethanol-Induced Gastric Damage in rats

4.8.1.Gross evaluation of gastric lesions

The anti-ulcer activity of *J. sambac* leaf extract in HCl/ethanol-induced gastric lesion model is shown in Figure 4.17. Results showed that rats pre-treated with omeprazole or *J. sambac* extract before being given HCl/ethanol solution had significantly reduced areas of gastric ulcer formation compared with ulcer control group. acidified ethanol solution produced extensive visible black hemorrhagic lesions of gastric mucosa. Moreover, this plant extract significantly suppressed the formation of the ulcers and it was interesting to note the flattening of gastric mucosal folds in rats pre-treated with the extract of this plant (500 mg/kg). It was also observed that protection of gastric mucosa was most prominent in rats pre-treated with 500 mg/kg leaf extract. The significant inhibition of gastric ulcer in

rats pretreated with *J. sambac* extract (250 mg/kg) was comparable with omeprazole which is a standard drug used for curing gastric ulcer. Similar results were observed in the stomach samples collected from the animals treated with *H. colorata* leaf extract at the highest dose used 400 mg/kg as shown in Figure 4.18.



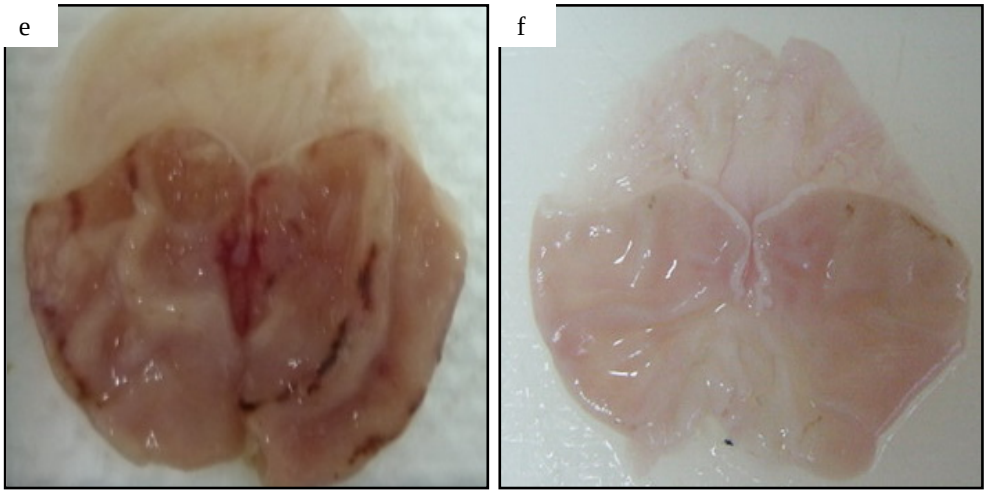
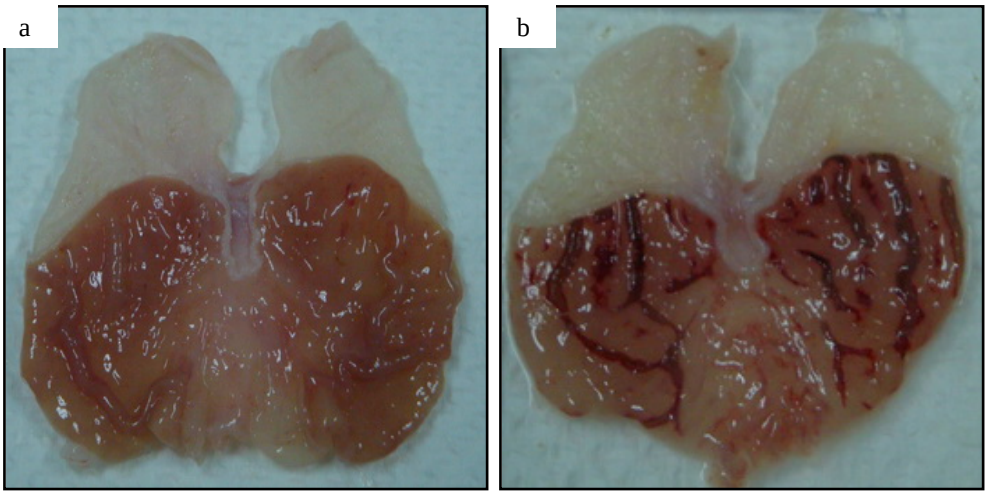


Figure 4.17: Gross appearance of the gastric mucosa in rats. (a) Rats treated with 5 ml/kg CMC (Normal control). No injuries to the gastric mucosa are seen. (b) Rats pre-treated with 5 ml/kg CMC (Ulcer control). Severe injuries are seen in the gastric mucosa. HCl/ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa. (c) Rats treated with omeprazole (20 mg/kg). Injuries to the gastric mucosa are milder compared to the injuries seen in the ulcer control rats. (d). Rats treated with *J. sambac* extract (62.50 mg/kg) showed moderate injuries are seen in the gastric mucosa. The extract reduces the formation of gastric lesions induced by acidified ethanol. (e). Rats reated with *J. sambac* extract (125 mg/kg) showed moderate gastric injuries. The extract reduces the formation of gastric lesions induced by acidified ethanol (f). Rats treated with *J. sambac* extract (250 mg/kg) showed mild gastric injuries. The extract reduces the formation of gastric lesions induced by acidified ethanol (g) Rats treated with 500 mg/kg of *J. sambac* extract. No injuries to the gastric mucosa are seen instead flattening of gastric mucosa is seen.



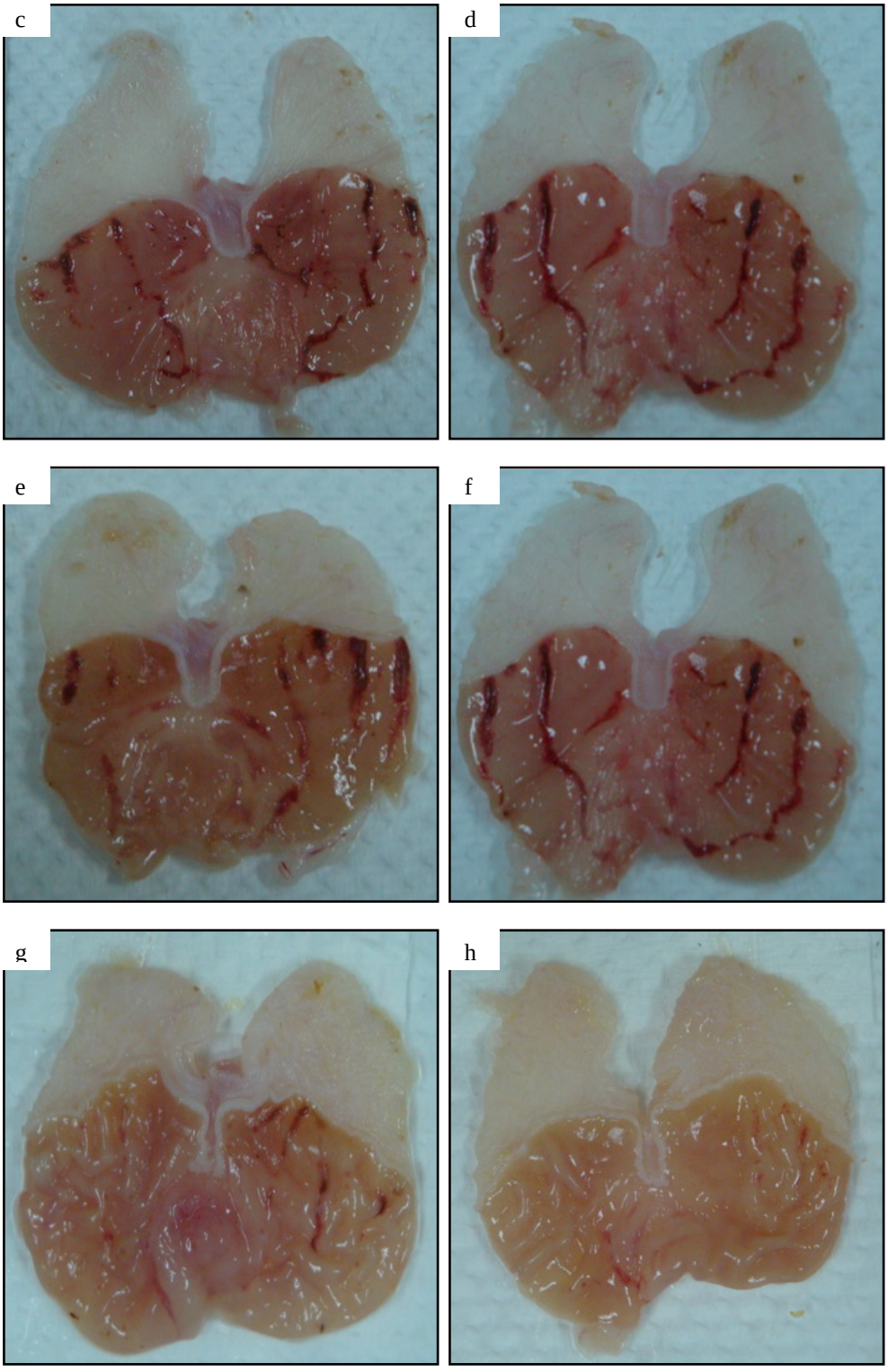
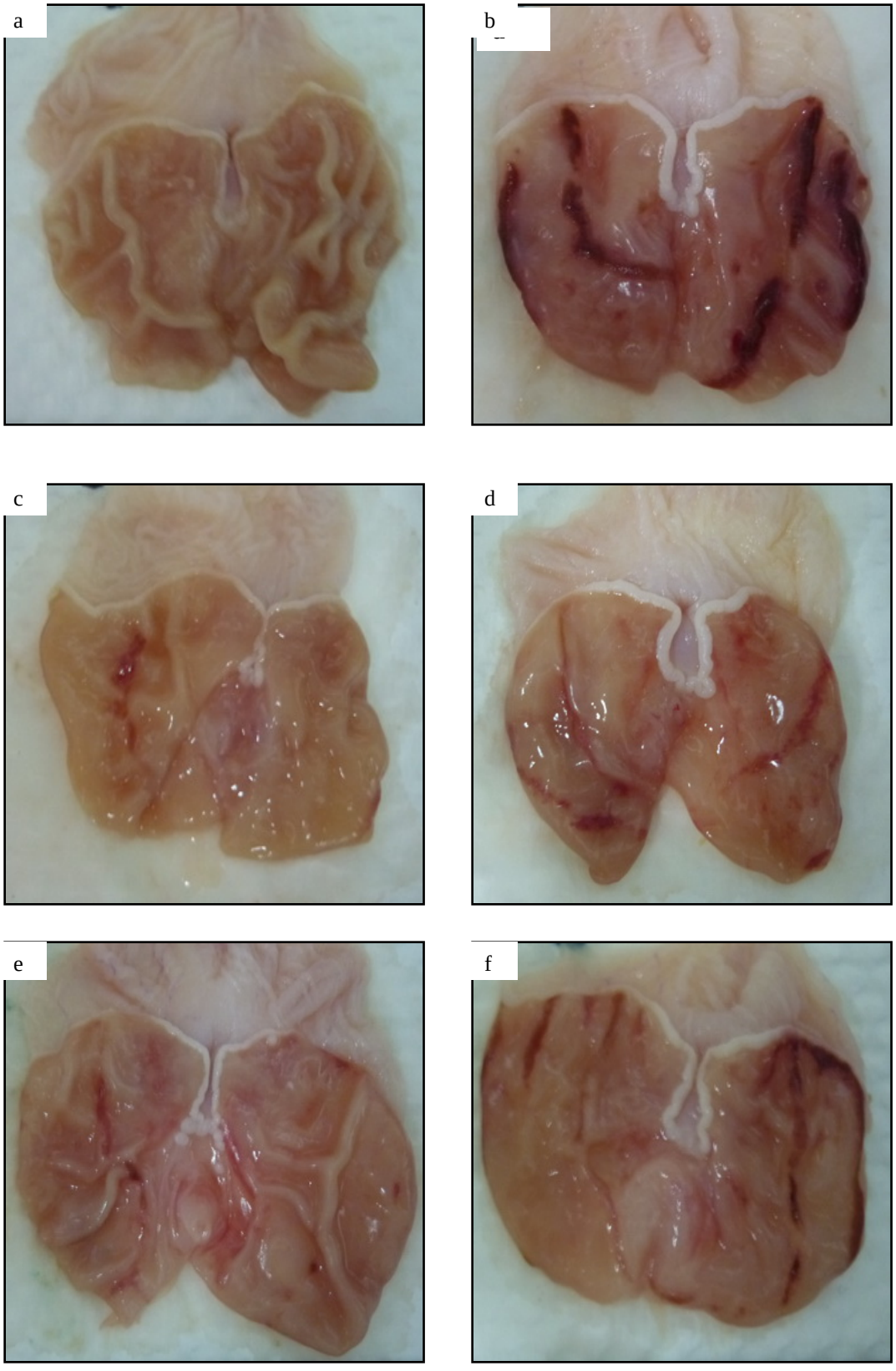


Figure 4.18: Gross appearance of the gastric mucosa in the rats treated with *H. colorata* leaf extract. (a) Rats pre-treated with 5 ml/kg CMC (Normal control). No injuries to the gastric mucosa are seen. (b) Rats pre-treated with 5 ml/kg CMC (Ulcer control). Severe injuries are seen in the gastric mucosa. HCl/ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa. (c) Rats pre-treated with omeprazole (20 mg/kg). Injuries to the gastric mucosa are milder compared to the injuries seen in the ulcer control rats. (d). Rat pre-treated with *H. colorata* extract (50 mg/kg). Moderate injuries are seen in

the gastric mucosa. The extract reduces the formation of gastric lesions induced by acidified ethanol. (e). Rat pre-treated with *H. colorata* extract (100 mg/kg). Moderate injuries are seen in the gastric mucosa. The extract reduces the formation of gastric lesions induced by acidified ethanol (f). Rat pre-treated with *J. sambac* extract (200 mg/kg). Mild injuries are seen in the gastric mucosa. The extract reduces the formation of gastric lesions induced by acidified ethanol (g) Rats pre-treated with 300 mg/kg of *J. sambac* extract. Minor injuries to the gastric mucosa are seen instead flattening of gastric mucosa is seen. (h) Rats pre-treated with 400 mg/kg of *J. sambac* extract. No injuries to the gastric mucosa are seen instead flattening of gastric mucosa is seen.

B-sitosterol administration to rats showed significant reduction in the areas of gastric ulcer formation compared with ulcer control group (Figure 4.19 and 4.20). Extensive visible black hemorrhagic lesions of gastric mucosa were observed in the ulcer control group due to the ulcerogenic effect of the acidified ethanol. β-sitosterol-treated groups showed flattening of gastric mucosal folds and more protection which is comparable to omeprazole-treated group than to the gastric mucosa treated with Octyl gallate. Figure 4.45 confirms these results from the calculation of the ulcer area which is significantly reduced in the low dose and high dose of β-sitosterol-treated groups (403.51 ± 14.44 and 264.53 ± 23.52 mm2 respectively) compared to the high areas obtained from the ulcer control group (915.17 ± 19.67 mm2) and the ulcer areas calculated from the low dose and high dose of Octyl gallate-treated groups (852.33 ± 21.08 and 169.71 ± 16.71 mm2 respectively).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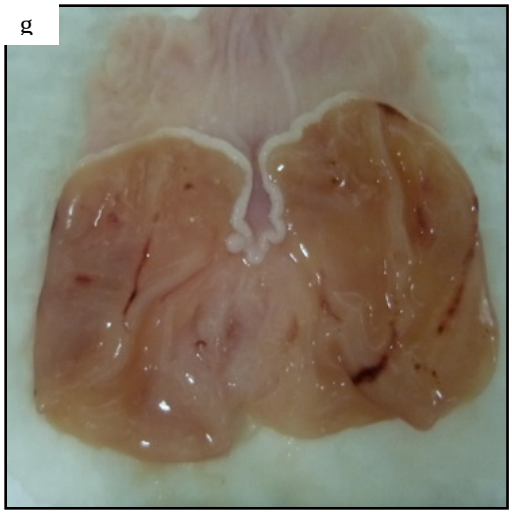


Figure 4.19: Gross appearance of the gastric mucosa in rats. (a) Rats pre-treated with 5 ml/kg CMC (Normal control). No injuries to the gastric mucosa are seen. (b) Rats pre-treated with 5 ml/kg CMC (Ulcer control). Severe injuries are seen in the gastric mucosa. HCl/ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa. (c) Rats pre-treated with omeprazole (20 mg/kg). Injuries to the gastric mucosa are milder compared to the injuries seen in the ulcer control rats. (d). Rat pre-treated with Low dose β-sitosterol compound (5 mg/kg). Moderate injuries are seen in the gastric mucosa. (e). Rat pre-treated with β-sitosterol compound (10 mg/kg). Moderate injuries are seen in the gastric mucosa. (f). Rat pre-treated with low dose Octyl gallate compound (5 mg/kg). Mild injuries are seen in the gastric mucosa. (g) Rats pre-treated with 10 mg/kg octyl gallate compound. No injuries to the gastric mucosa are seen instead flattening of gastric mucosa is seen.

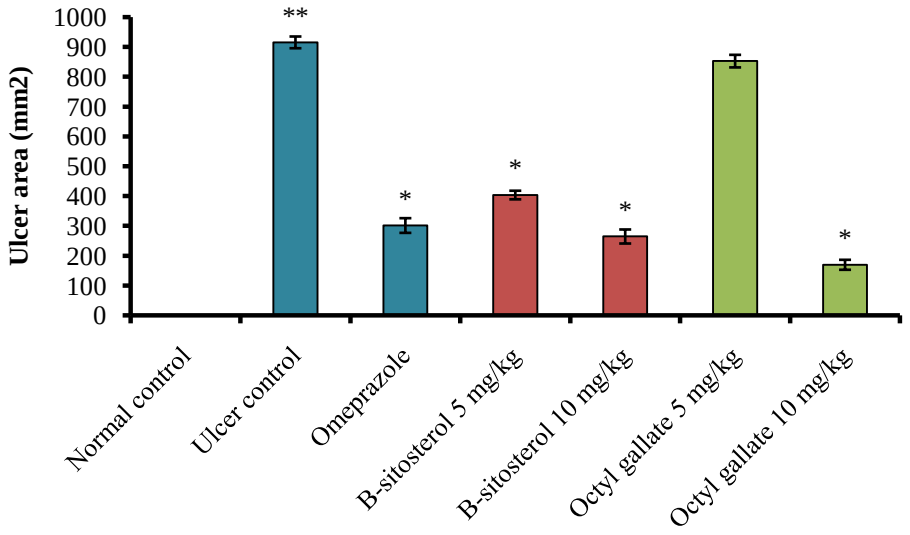


Figure 4.20: The effect of β-sitosterol and Octyl gallate treatment on the ulcer area of the animals from all groups. Data are presented in the form of Mean ± SEM. ***P*<0.001 compared to normal control group. **P*<0.001 compared to ulcer control group.

4.8.2. Ulcer Area and Inhibition

The effect of *J.* sambac and *H. colorata* leaf extracts on the ulcer area due to HCl/Ethanol are shown on Figures 4.21 and 4.22 respectively. The results revealed significant reduction (*P*<0.001) in the highest dose *J. sambac*-treated rats (500 mg/kg) (0.01 ± 0.00 mm2) showing 100 % inhibition compared to the high value recorded from the ulcer control group animals (734.02 ± 29.16 mm2). In addition, the medium dose of *J. sambac* 125 mg/kg recorded similar inhibition to that of omeprazole-treated animals (77.83 and 77.06% respectively). Further, treating the animals with the different doses of *H. colorata* showed significant decrease in the ulcer area compared with ulcer control group animals and the high dose *H. colorata*-treated group recorded 25.34 ± 2.60 mm2 inhibiting the ulcer area by 97.19% whereas, ulcer control group which recorded 902.00 ± 12.60 mm2 ulcer area.

These results indicate that both plant extracts have anti-ulcer activity depending on the dose used.

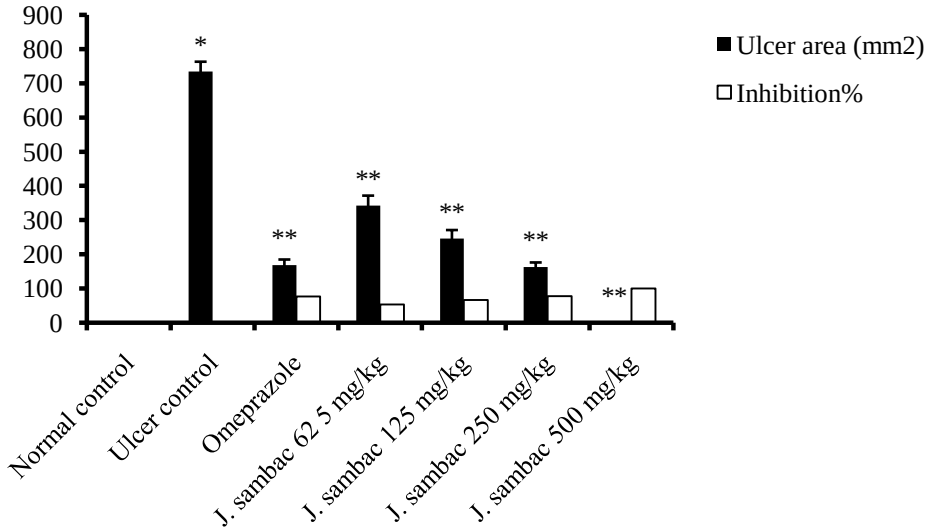


Figure 4.21: Effect of *J. sambac* leaf extract on the ulcer area and the inhibition% calculated from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.001 compared to normal control. ***P*<0.001 compared to ulcer control.

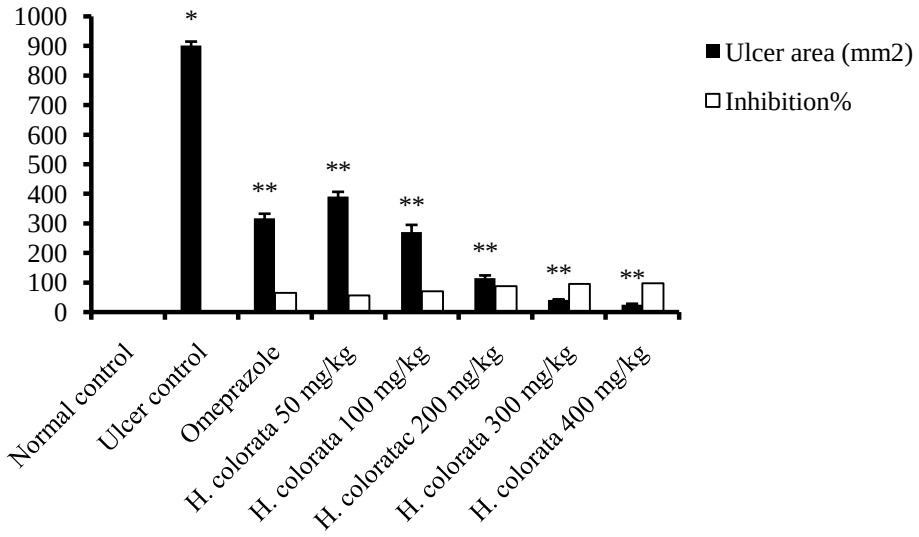


Figure 4.22: Effect of *H. colorata* leaf extract on the ulcer area and the inhibition% calculated from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal control. ***P*<0.05 compared to ulcer control.

4.8.3. Gastric Wall Mucosal content (GWM) Evaluation

Treatment with HCl/ethanol caused a significant decrease in the mucus content of the gastric wall in the untreated animals (ulcer control group) (Figure 4.23 and 4.24). The depleted gastric mucus was significantly replenished after pre-treatment with the *J. sambac* and *H. colorata* extracts. It was also found that the pre-treatment with *J. sambac* at doses of 62.5, 125, 250 and 500 mg/kg significantly increased the amount of gastric mucus in the acidified ethanol-ulcerated rats (Figure 23) reaching 694.09 ± 13.09 mg Alcian blue/g tissue compared to the low value from the ulcer control group (296.67 ± 9.83 mg Alcian blue/g tissue) and approaching the record from the omeprazole-treated group animals (690.40 ± 8.77 mg Alcian blue/g tissue). Similar results observed from the highest dose of *H. colorata* treatment (508.33 ± 24.24 mg Alcian blue/g tissue) compared to the ulcer control group (126.17 ± 24.96 mg Alcian blue/g tissue) (Figure 4.24). The results show significant protection of both plant extracts to the gastric mucosal wall.

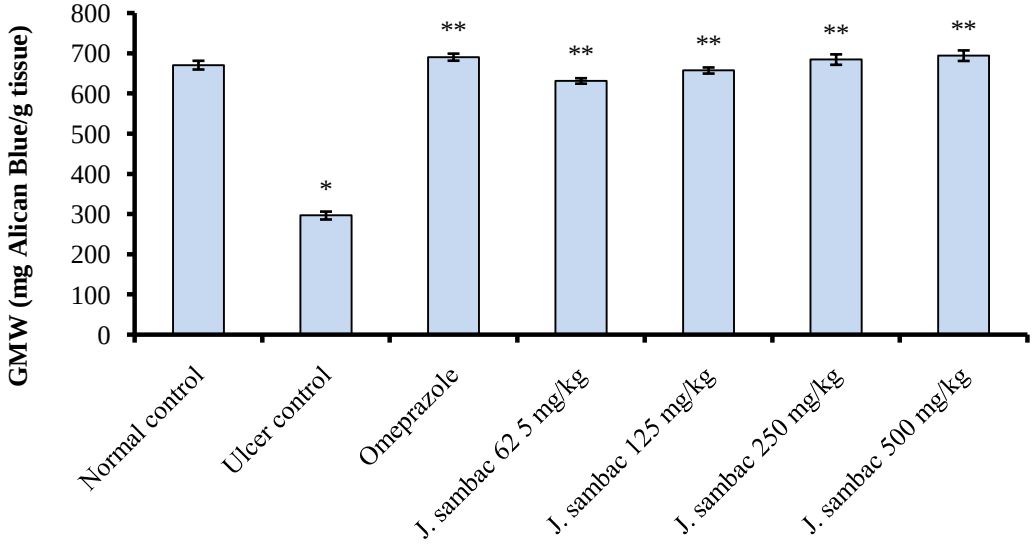


Figure 4.23: Effect of *J. sambac* leaf extract on the gastric wall mucosal calculated from the rats of all the experimental groups. All values are expressed as Mean ± SEM. **P*<0.05 compared to normal control. ***P*<0.05 compared to ulcer control.

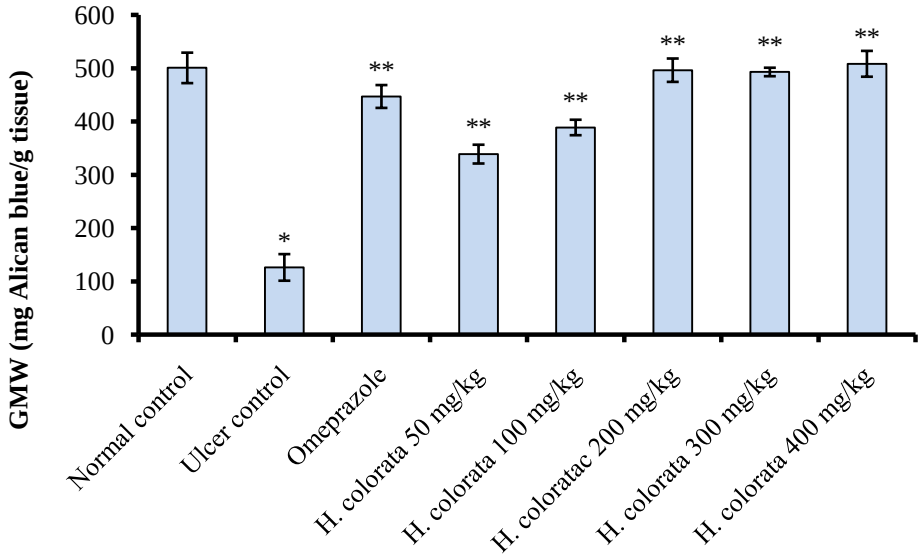


Figure 4.24: Effect of *H. colorata* leaf extract on the gastric wall mucosal calculated from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal control. ***P*<0.05 compared to ulcer control.

Adminstration of HCl/Ethanol to the rats reduced the gastric wall mucus to 108.50 ± 8.59 mg Alican blue/g tissue compared to normal group (413.83 ± 15.40 mg Alican blue/g tissue). Pre-treatment of the rats with low and high dose β-sitosterol significantly improved the mucus secretion inside the stomachs of animals reaching 232.17 ± 15.40 and 347.00 ± 18.42 mg alican blue/g tissue respectively, whereas pre-treatment of the animals with Octyl gallate showed only significance (*P*<0.001) in the high dose-treated group rats (340.43 ± 13.29 mg Alican blue/g tissue) as shown in Figure 4.25.

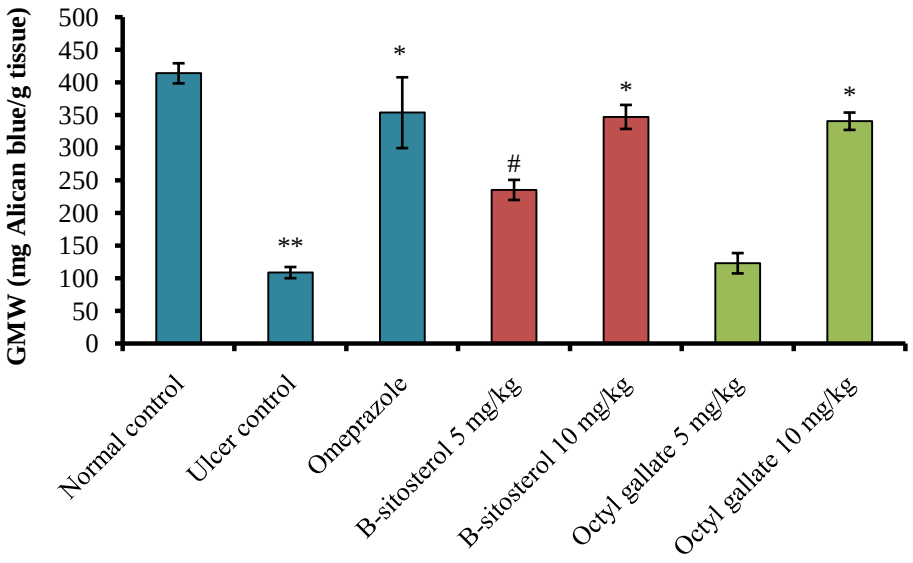


Figure 4.25: The effect of β-sitosterol and Octyl gallate treatment on the gastric wall mucus of the animals from all groups. Data are presented in the form of Mean ± SEM. ***P*<0.05 compared to normal group. **P*<0.05 compared to ulcer group.

4.8.4. pH of Gastric Content of *J. sambac* and *H. colorata* crude extracts

The acidity of gastric content in experimental animals pretreated with omeprazole, *J. sambac* and *H. colorata* leaf extract were decreased significantly (*P*<0.05) in the high dose of each plant extract compared with that of the ulcer control group. The high dose *J. sambac* and *H. colorata*-treated animals had higher pH value (5.37 ± 0.15 and 5.03 ± 0.05 respectively) compared to their ulcer control groups (3.19 ± 0.15 and 3.08 ± 0.19 respectively) as shown in Figures 4.26 and 4.27. These results confirm the gastroprotective activity of both plant extracts to the gastric acidity.

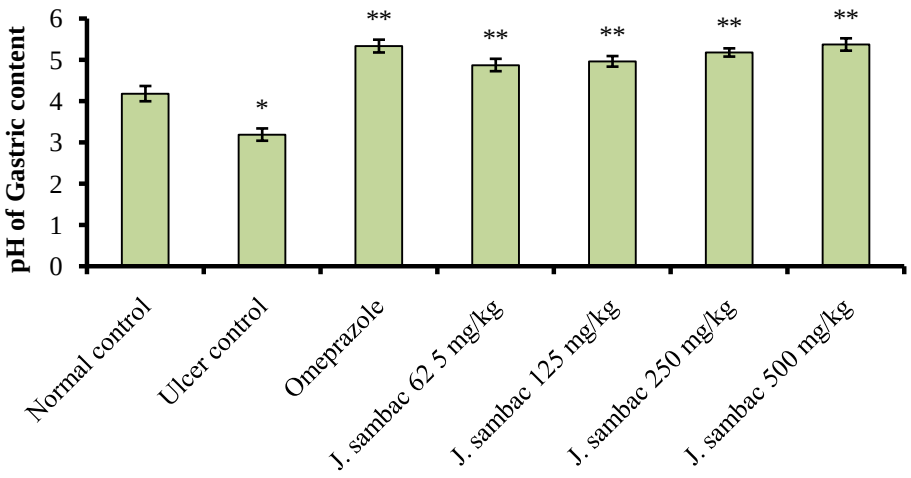


Figure 4.26: Effect of *J. sambac* leaf extract on the gastric pH determined from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal control. ***P*<0.05 compared to ulcer control.

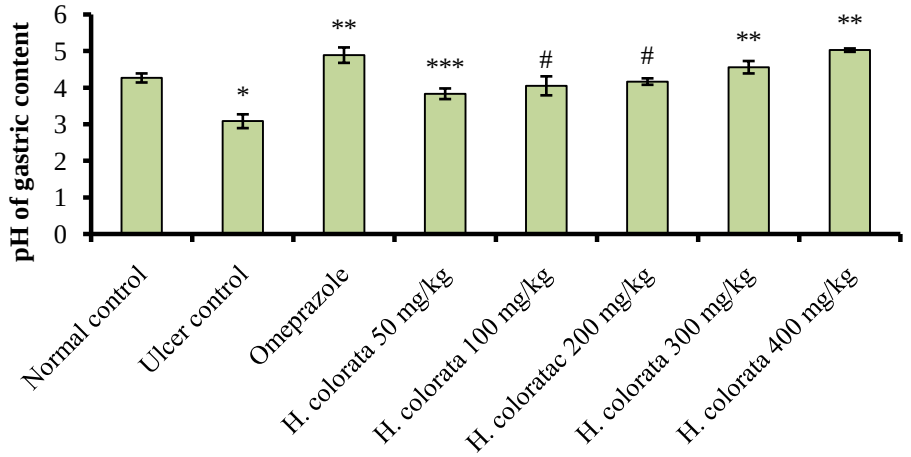


Figure 4.27: Effect of *H.Colorata* leaf extract on the gastric pH determined from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal control. ***P*<0.05 compared to ulcer control.

The effect of β-sitosterol and Octyl gallate treatment on the gastric acidity of the animals from all groups is shown in Figure 4.28. High dose β-sitosterol-treated rats (10 mg/kg) showed significant (P<0.05) increase in pH (4.50 ± 0.26) similar to omeprazole-treated animals (4.46 ± 0.21) and compared to ulcer control group (2.87 ± 0.26) and low dose β-sitosterol-treated animals (3.73 ± 0.22). On the contrary, the low dose and the high dose Octyl gallate treatment didn't show significant improvement in the gastric pH. From the results, β-sitosterol is more effective than Octyl gallate in reducing the gastric acidity due to HCL/ethanol administeration.

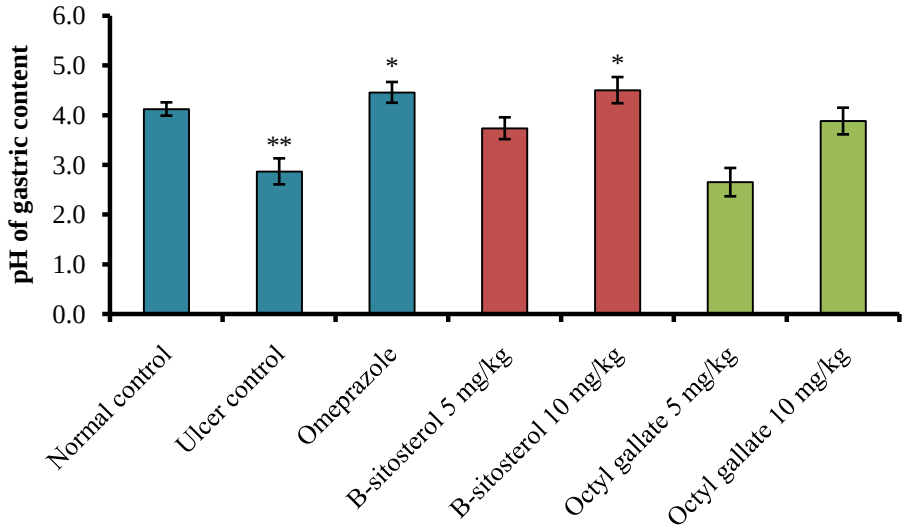
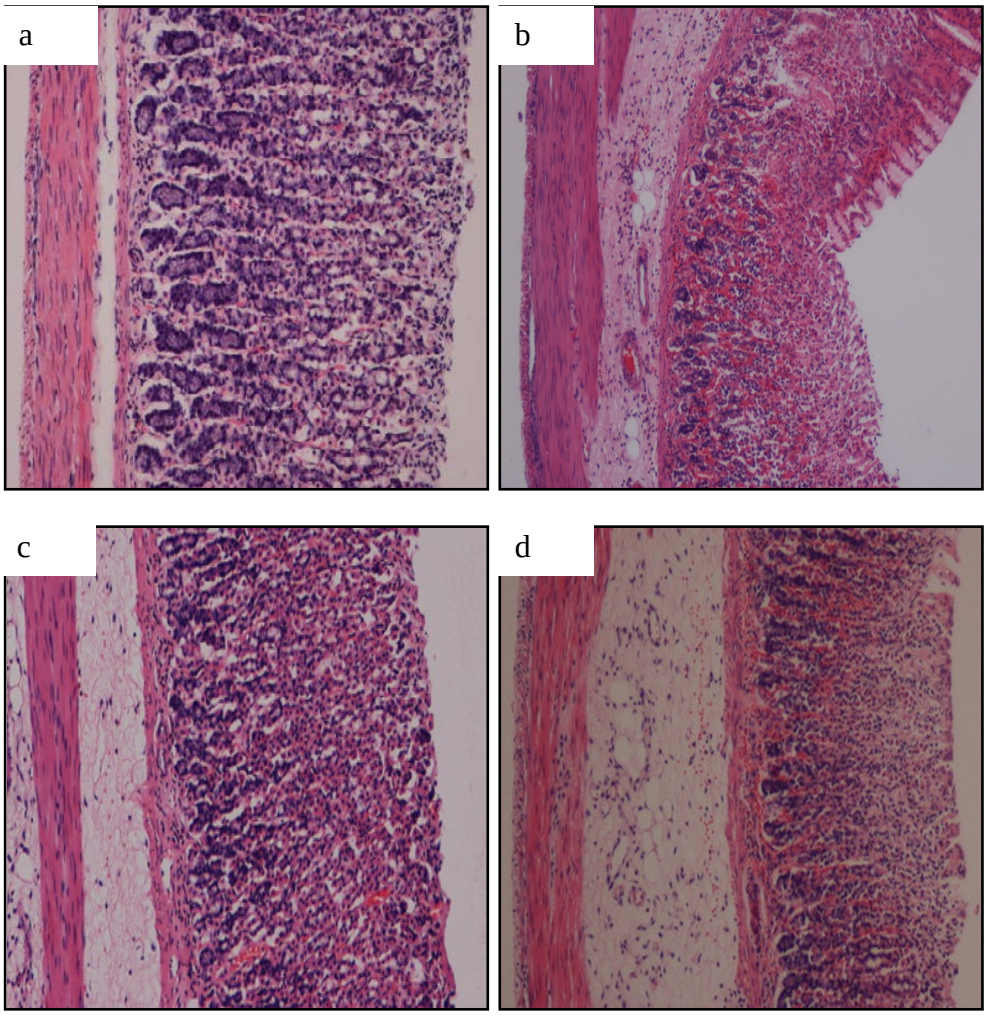


Figure 4.28: The effect of β-sitosterol and Octyl gallate treatment on the gastric acidity of the animals from all groups. Data are presented in the form of Mean ± SEM. **P<0.05 compared to normal group. *P<0.05 compared to ulcer group.

4.8.5. Histological Evaluation of Gastric Lesions

Histological observation of HCl/ethanol induced gastric lesions in ulcer control group showed comparatively extensive damage to the gastric mucosa and necrotic lesions penetrate deeply into mucosa, and extensive oedema and leucocytes infiltration of the submucosal layer are present (Figures 4.29 and 4.30). Rats that received pre-treatment with *J. Sambac* and *H. colorata* extracts had comparatively better protection of the gastric mucosa as seen by reduction of ulcer area, reduced of submucosal oedema and leucocytes infiltration. These results confirm the previous findings for the anti-ulcer effect of both plant extracts.



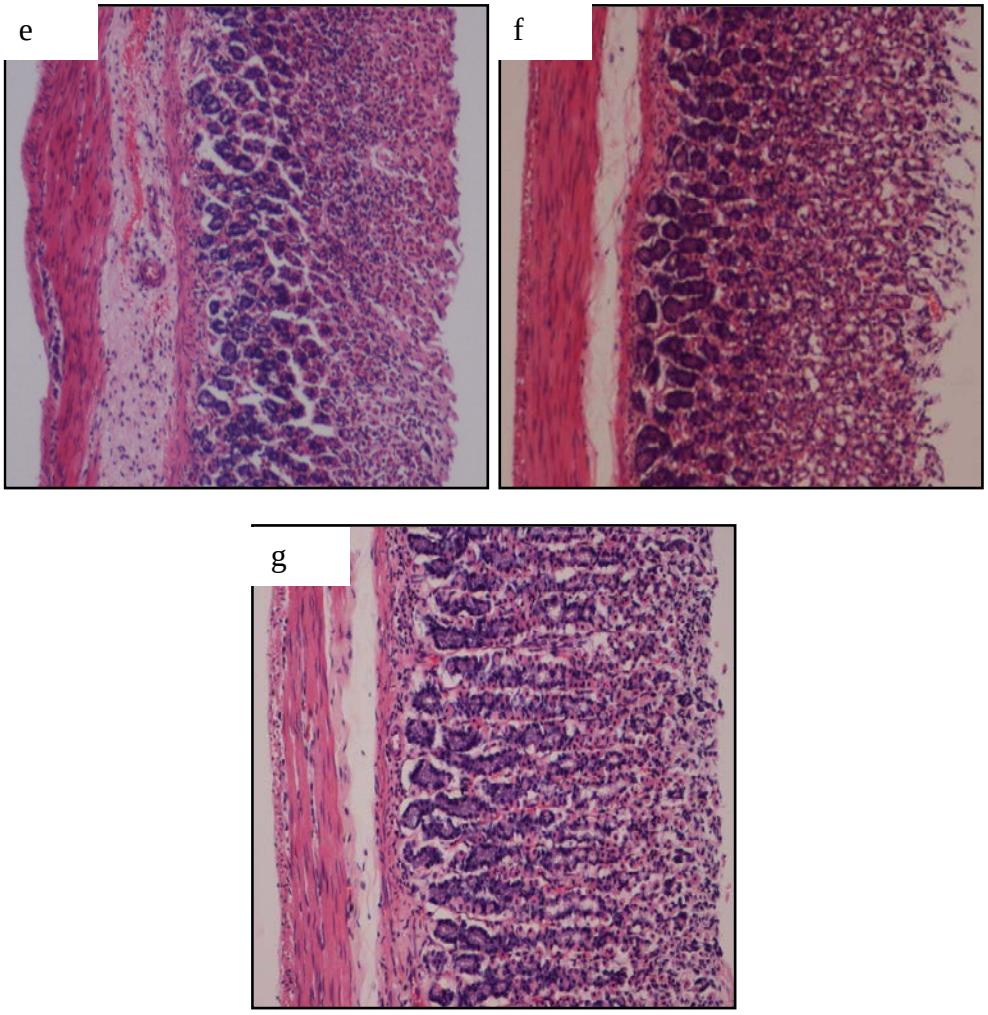
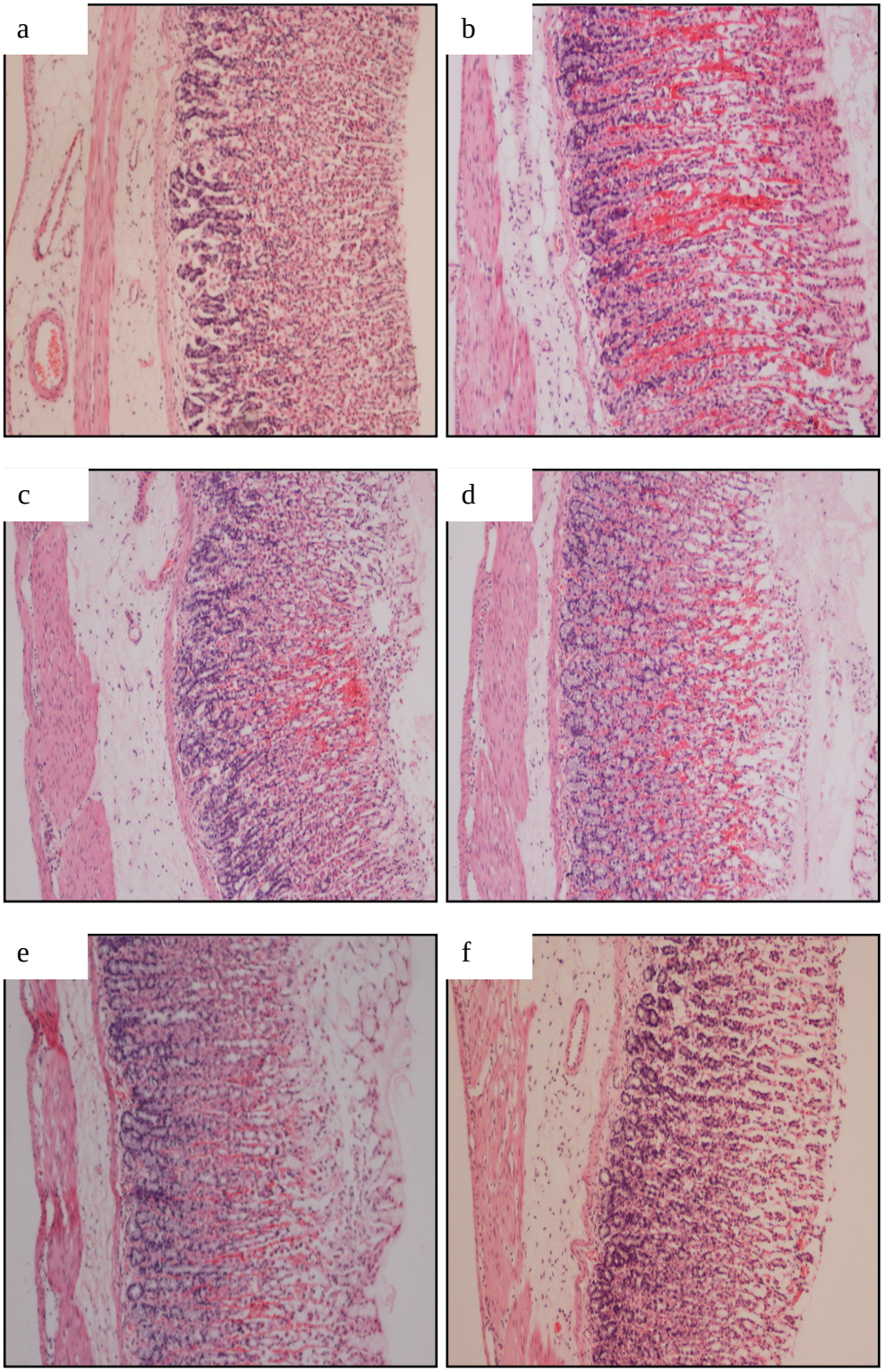


Figure 4.29: Histological study of HCl/ethanol-induced gastric mucosal damage in rats. (a) Rats pre-treated with 5 ml/kg of CMC (Normal control group). No injuries to the gastric mucosa are seen (b) Rats pre-treated with 5 ml/kg of CMC (ulcer control group). There is severe disruption to the surface epithelium and necrotic lesions penetrating deeply into mucosa and extensive edema of submucosa layer and leucocytes infiltration is present. (c) Rats pre-treated with omeprazole (20 mg/kg). Mild disruption of the surface epithelium mucosa is seen. There is edema and leucocytes infiltration of the submucosal layer. (d) Rat pre-treated with *J. sambac* extract (62.50 mg/kg). Moderate disruption of surface epithelium is present. There is submucosal edema and leucocytes infiltration. (e) Rats pre-treated with *J. sambac* extract (125 mg/kg). There is mild disruption to the surface epithelium. There is edema with leucocytes infiltration of the submucosal layer. (f). Rats pre-treated with *J. sambac* extract (250 mg/kg). There is mild disruption to the surface epithelium. There is no edema or leucocytes infiltration of the submucosal layer (g).Rats pre-treated with *J. sambac* extract (500 mg/kg). There is no disruption to the surface epithelium and no edema or leucocytes infiltration of the submucosal layer (H & E stain 10x).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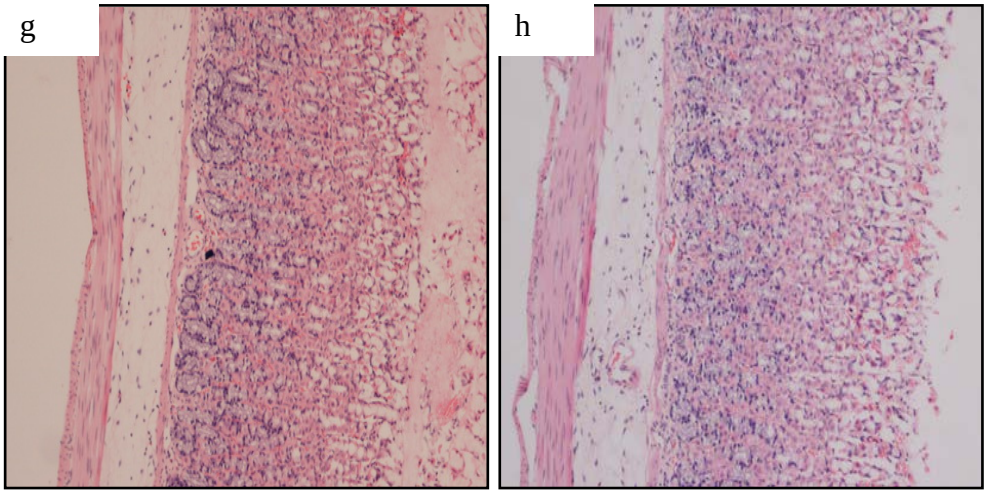
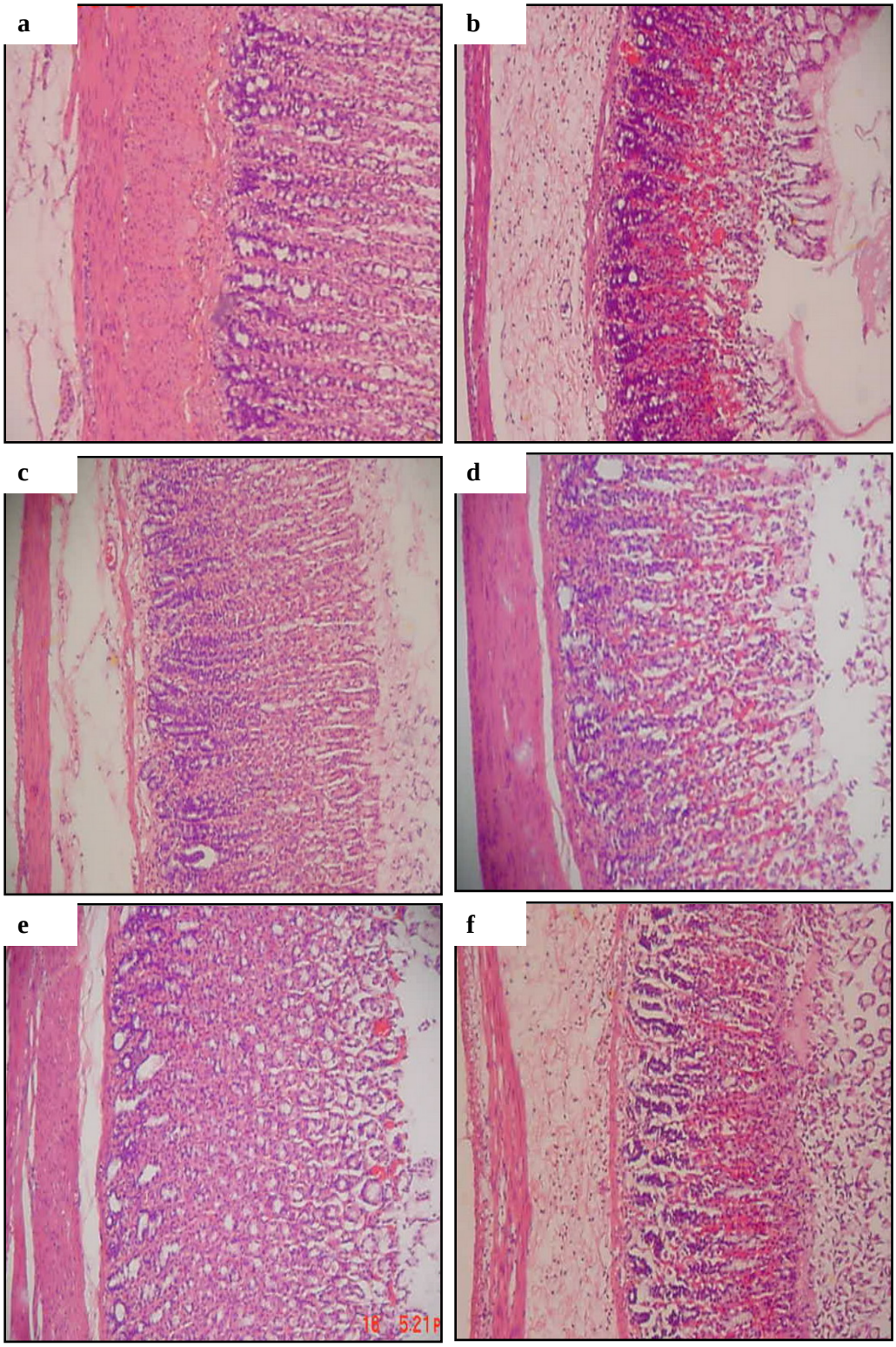


Figure 4.30: Histological study of HCl/ethanol-induced gastric mucosal damage in rats. (a) Rats pre-treated with 5 ml/kg of CMC (Normal control group). No injuries to the gastric mucosa are seen (b) Rats pre-treated with 5 ml/kg of CMC (ulcer control group). There is severe disruption to the surface epithelium and necrotic lesions penetrating deeply into mucosa and extensive edema of submucosa layer and leucocytes infiltration is present. (c) Rats pre-treated with omeprazole (20 mg/kg). Mild disruption of the surface epithelium mucosa is seen. There is edema and leucocytes infiltration of the submucosal layer. (d) Rat pre-treated with *H. colorata* extract (50 mg/kg). Moderate disruption of surface epithelium is present. There is submucosal edema and leucocytes infiltration. (e) Rats pre-treated with *H. colorata* extract (100 mg/kg). There is mild disruption to the surface epithelium. There is edema with leucocytes infiltration of the submucosal layer. (f). Rats pre-treated with *H. colorata* extract (200 mg/kg). There is mild disruption to the surface epithelium. There is no edema or leucocytes infiltration of the submucosal layer (g and h).Rats pre-treated with *H. colorata* extract (300 and 400 mg/kg respectively). There is no disruption to the surface epithelium and no edema or leucocytes infiltration of the submucosal layer (H & E stain 10x).

Sections of the stomach samples collected from ulcer control group showed comparatively extensive damage to the gastric mucosa and severe necrotic lesions with extensive oedema and leucocytes infiltration of the submucosal layer. Pre-treatment of rats with β-sitosterol compound showed comparatively better protection of the gastric mucosa than sections of Octyl gallate-treated animals as seen by reduction of ulcer area, reduced of submucosal oedema and leucocytes infiltration (Figure 4.31).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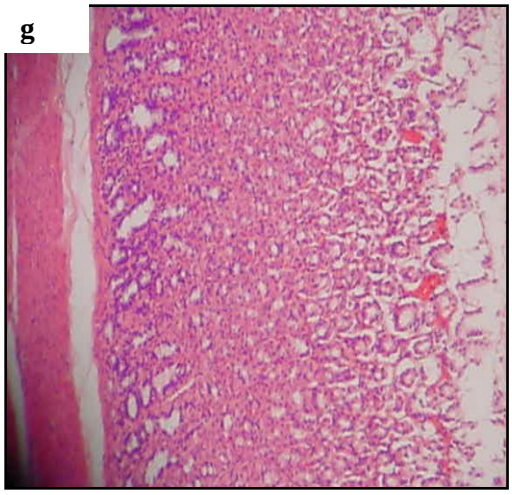


Figure 4.31: Histological study of HCl/ethanol-induced gastric mucosal damage in rats. (a) Rats pre-treated with 5 ml/kg of CMC (Normal control group). No injuries to the gastric mucosa (b) Rats pre-treated with 5 ml/kg of CMC (ulcer control group). There is severe disruption to the surface epithelium and necrotic lesions and extensive edema of submucosa layer and leucocytes infiltration is present. (c) Rats pre-treated with omeprazole (20 mg/kg). Mild disruption of the surface epithelium mucosa is seen. There is edema and leucocytes infiltration of the submucosal layer. (d) Rat pre-treated with β-sisterol (5 mg/kg) showed moderate damage in the surface epithelium, submucosal edema and infiltration of leucocytes. (e) Rats treated with β-sitosterol (10 mg/kg) showed mild damage in the surface epithelium with minor leucocytes infiltration of the submucosal layer. (f). Rats treated with Octyl gallate (5 mg/kg) showed considerable damage in the surface epithelium, edema and leucocytes infiltration (g).Rats treated with Octyl gallate (10 mg/kg) showed less damage in the surface epithelium without edema or leucocytes infiltration in the submucosal layer (H & E stain 10x).

4.8.6. Immunohistochemistry of Hsp70 and Bax

Immunohistochemical results demonstrated that treatment of HCl/ethanol induced injury rats with *J. Sambac* and *H. colorata* extracts induced over-expression of Hsp70 protein (Figures 4.32 and 4.33). In addition to this, the expression of Hsp70 protein in normal control and HCl/ethanol-induced gastric tissues (ulcer control group) was found to be down-regulated compared to Hsp70 expression in *J. Sambac* and *H. colorata* extracts-treated groups. Immunohistochemical staining of Bax protein demonstrated that pre-

treatment of HCl/ethanol-induced injury rats with *J. Sambac* extract caused down-expression of Bax protein. In addition to this, the expression of Bax in HCl/ethanol-induced gastric tissues (ulcer control group) was found to be up-regulated compared to *J. Sambac* and *H. colorata*-treated group.

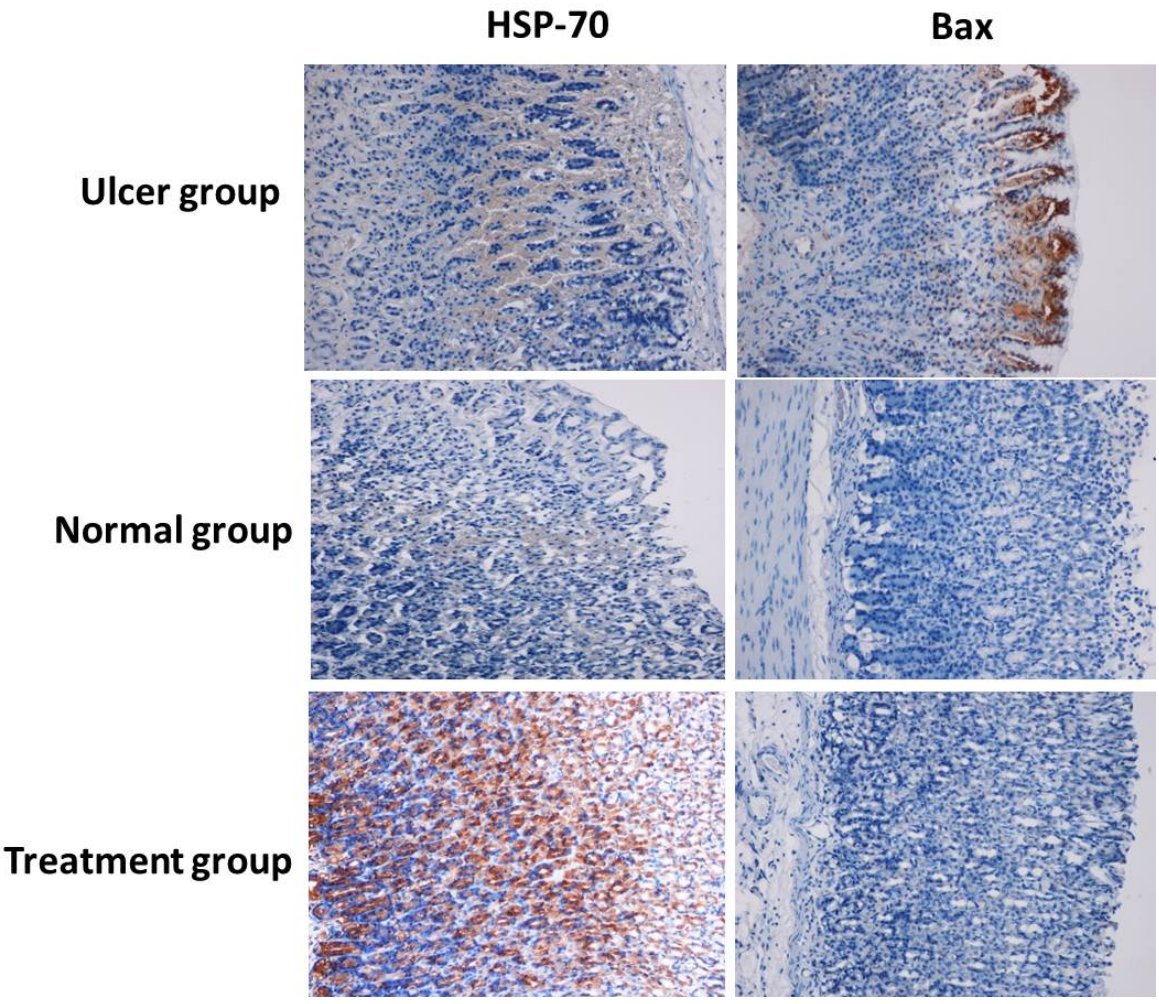


Figure 4.32: Immunohistochemical analysis of expression of Hsp and Bax proteins in the stomach of rats in HCl/ethanol-induced gastric ulcer. Immunohistochemistry staining of Hsp70 and Bax proteins showed over-expression of Hsp70 protein and down-expression of Bax protein in rats pre-treated with *J. sambac* leaf extract (Magnification 10x).

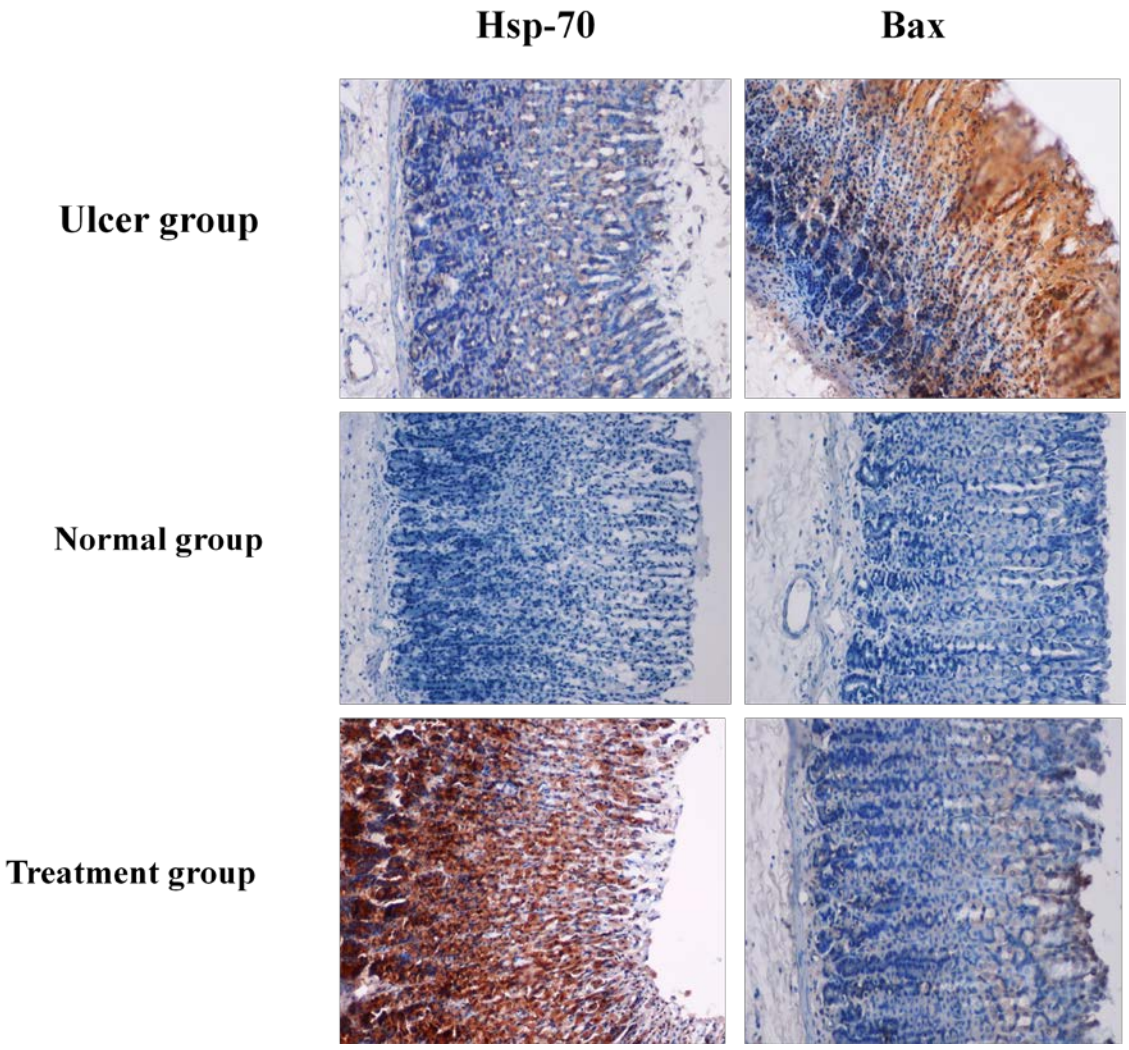
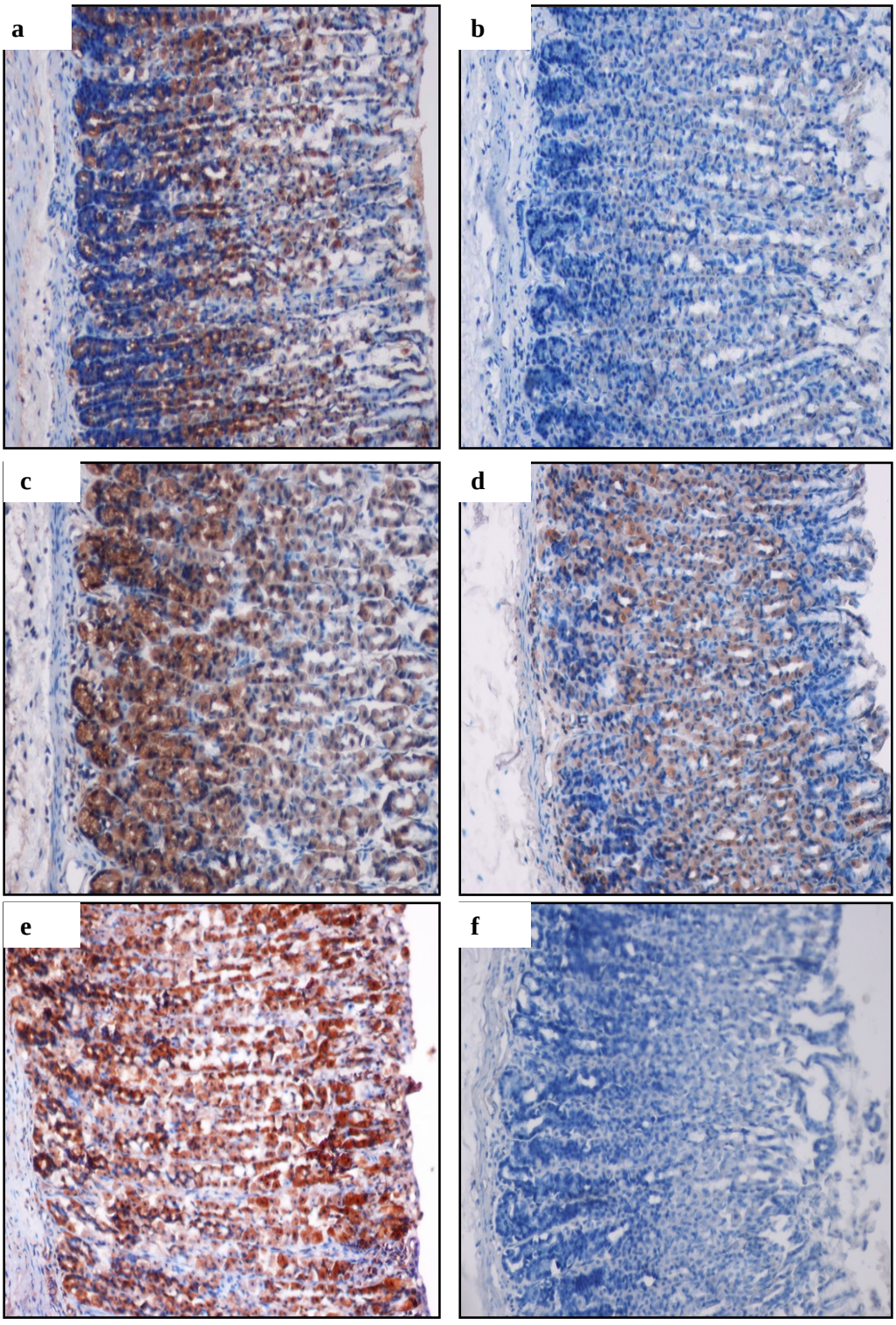


Figure 4.33: Immunohistochemical analysis of expression of Hsp and Bax proteins in the stomach of rats in HCl/ethanol-induced gastric ulcer. Immunohistochemistry staining of Hsp70 and Bax proteins showed over-expression of Hsp70 protein and down-expression of Bax protein in rats pre-treated with *H. colorata* leaf extract (Magnification 10x).

Immunohistochemical results demonstrated that pre-treatment of rats with β-sitosterol caused over-expression of Hsp70 protein (Figure 4.34) significantly better than Octyl gallate treatment confirming the suggestion that β-sitosterol gives better protection to the stomach in the same manner as omeprazole and better than Octyl gallate treatment. In addition to this, the expression of Hsp70 protein in normal control and HCl/ethanol-

induced gastric tissues (ulcer control group) was found to be down-regulated compared to Hsp70 expression in *J. Sambac* extract-treated group.



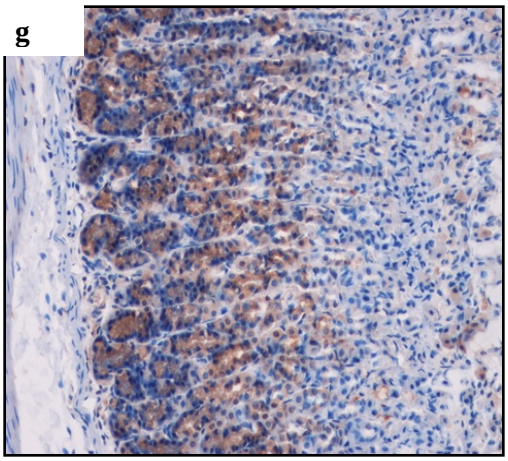


Figure 4.34: Immunohistochemical analysis of expression of Hsp protein in the stomach of rats (a) Rats treated with 5 ml/kg of CMC showing down-regulation of Hsp70 (b) Rats treated with 5 ml/kg of CMC revealed severe damage with no expression of Hsp70 (c) Rats treated with omeprazole (20 mg/kg) showed minor damage in the surface epithelium with over-expression of Hsp70. (d) Rat treated with β-sisterol (5 mg/kg) showed moderate damage with mild expression of Hsp70 (e) Rats treated with β-sitosterol (10 mg/kg) showed minor damage in the surface epithelium with over-expression of Hsp70 (f). Rats treated with Octyl gallate (5 mg/kg) showed considerable damage in the surface epithelium and no expression of Hsl70 (g).Rats treated with Octyl gallate (10 mg/kg) revealed moderate damage in the surface epithelium and moderate expression of Hsp70 (Magnification 20x).

4.8.7. MDA Level in the Gastric Tissue Homogenate

Administration of HCl/ethanol significantly increase the MDA level of gastric homogenate in ulcer control group compared with normal control. The effect of *J. sambac* and *H. colorata* leaf extracts on the MDA level in the gastric tissue homogenate from all experimental groups' animals is shown in Figures 4.35 and 4.36. The different doses of *J.sambac* and *H. colorata* extracts used in the experiments showed significant reduction in the level of MDA in the gastric tissue homogenates compared to ulcer control group. The level of MDA in the high dose *J. sambac*-treated group animals was significantly low (27.05 ± 1.87 μmol/ mg protein) compared to its ulcer control (93.89 ± 4.86 μmol/mg

protein) and its omeprazole-treated group (41.25 ± 2.39 µmol/mg protein). Moreover, the high dose *H. colorata*-treated group animals showed significant reduction in the MDA level of the gastric tissue homogenate (107.52 ± 4.58 µmol/mg protein) compared to ulcer control and omeprazole-treated group (267.14 ± 16.56 and 169.11 ± 14.54 µmol/mg protein respectively). The results propose the efficacy of both plant extracts against HCl/ethanol-induced gastric damage.

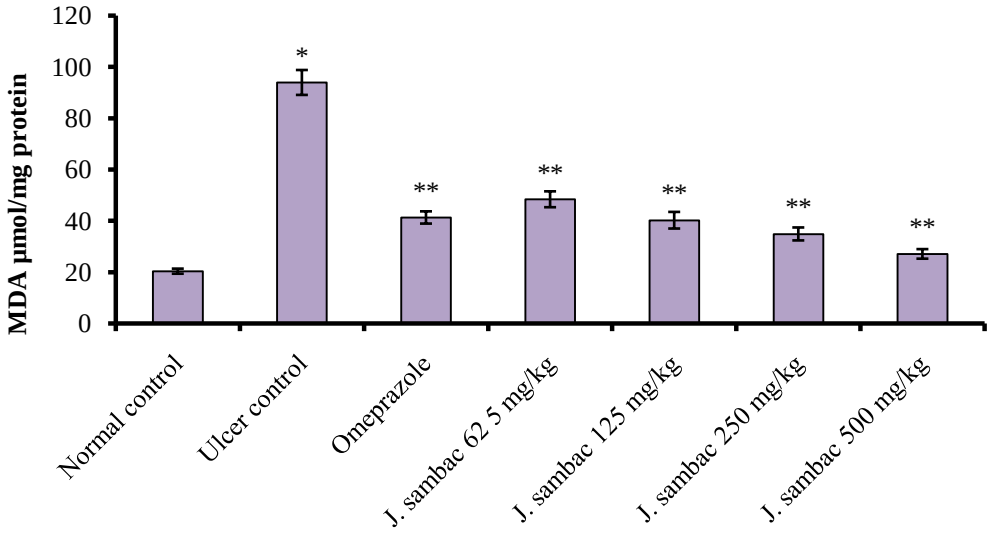


Figure 4.35: Effect of *J. sambac* leaf extract on the level of MDA in the gastric tissue homogenate collected from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal control. ***P*<0.05 compared to ulcer control.

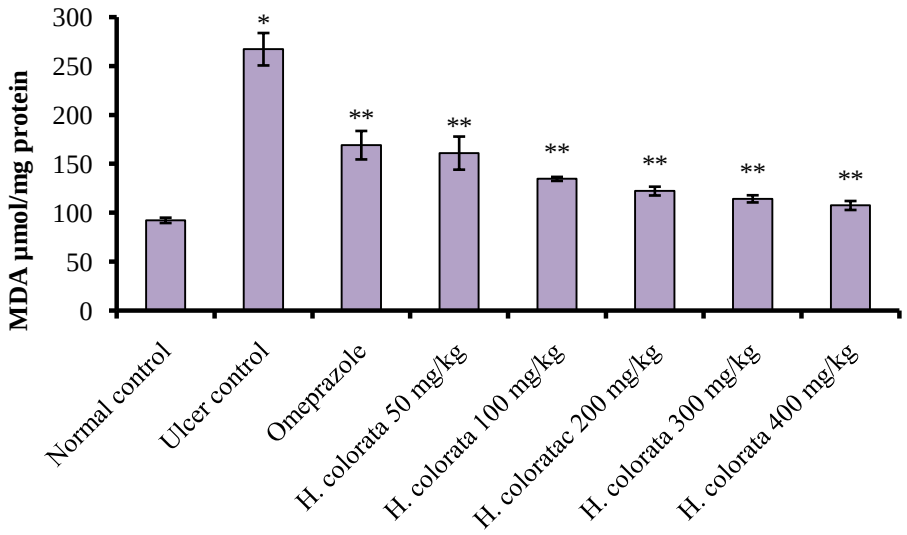


Figure 4.36: Effect of *H. colorata* leaf extract on the level of MDA in the gastric tissue homogenate collected from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal group. ***P*<0.05 compared to ulcer group.

4.8.8. Enzymatic Activity in the Gastric Tissue Homogenate

In the gastric tissue homogenate, both PGE_2 and SOD activities in ulcer control group were significantly lower compared with normal control group in both plant extracts *J. sambac* and *H. colorata* (Figures 4.37 and 4.38). Administration of omeprazole or *J. sambac* before HCl/ethanol significantly elevated level of PGE_2 and SOD compared with ulcer control group. The level of PGE2 and SOD in case of high dose *J. sambac* extract treatment (500 mg/kg) increased to 4.02 ± 0.17 ng/mg protein and 455.83 ± 10.41 U/mg protein respectively compared with ulcer control (1.15 ± 0.05 ng/mg protein and 322.35 ± 3.29 U/mg protein respectively) and higher than omeprazole-treated group (4.58 ± 0.22 ng/mg protein and 486.00 ± 3.58 U/mg protein respectively) (Figure 4.37a, b). As for *H. colorata* treatment, the level of PGE2 and SOD reached in the high dose-treated group 4.91 ± 0.07 ng/mg protein and 430.18 ± 12.48 U/mg protein respectively which is approximately similar to the results obtained from the omeprazole-treated group animal (4.78 ± 0.06

ng/mg protein and 411.47 ± 14.85 U/mg protein respectively) and compared with the ulcer control group animals (1.08 ± 0.02 ng/mg protein and 235.39 ± 25.49 U/mg protein respectively. Our results recommend *J. sambac* and *H. colorata* leaf extracts as potential antiulcer.

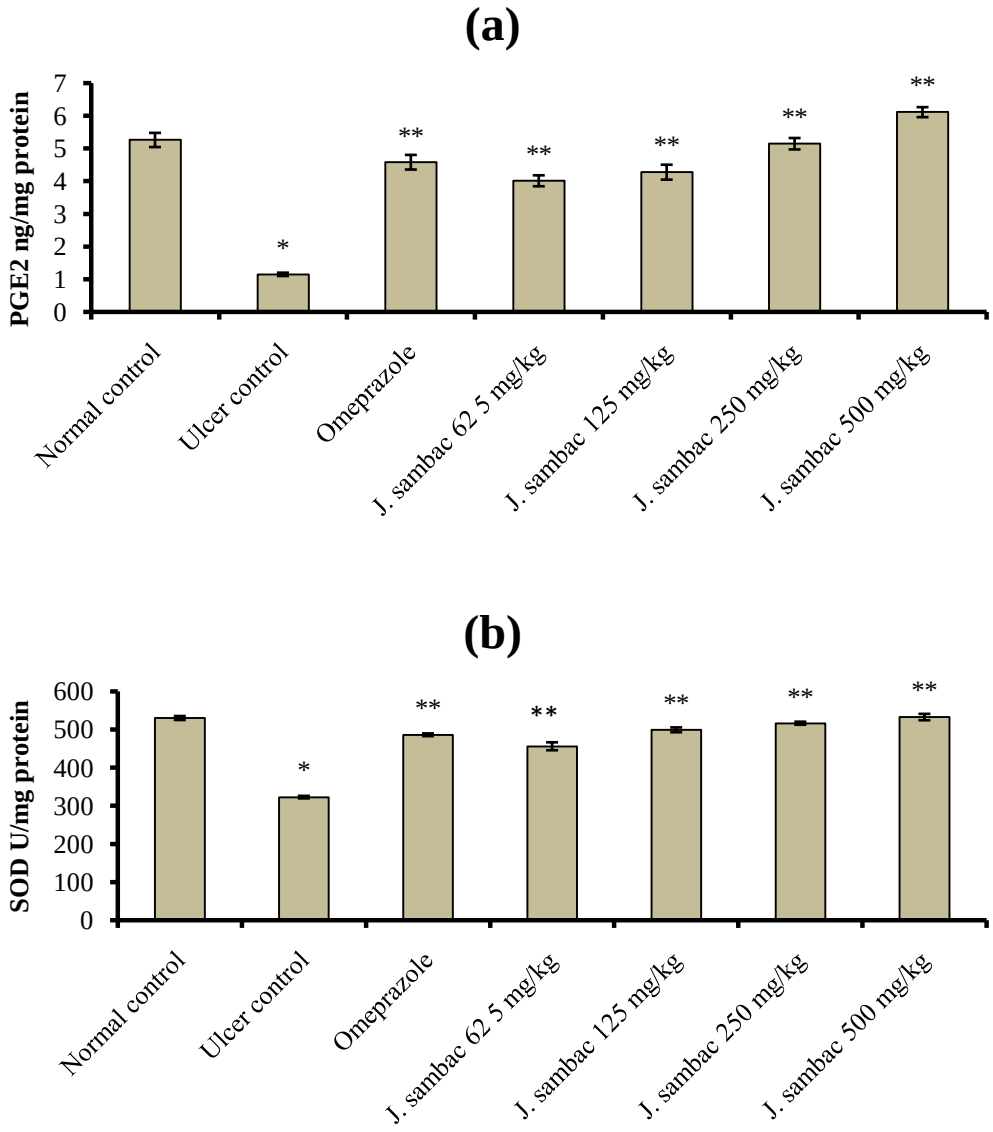


Figure 4.37a, b: Effect of *J. sambac* leaf extract on the level of **(a)** PGE2 and **(b)** SOD in the gastric tissue homogenate collected from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal group. ***P*<0.05 compared to ulcer group.

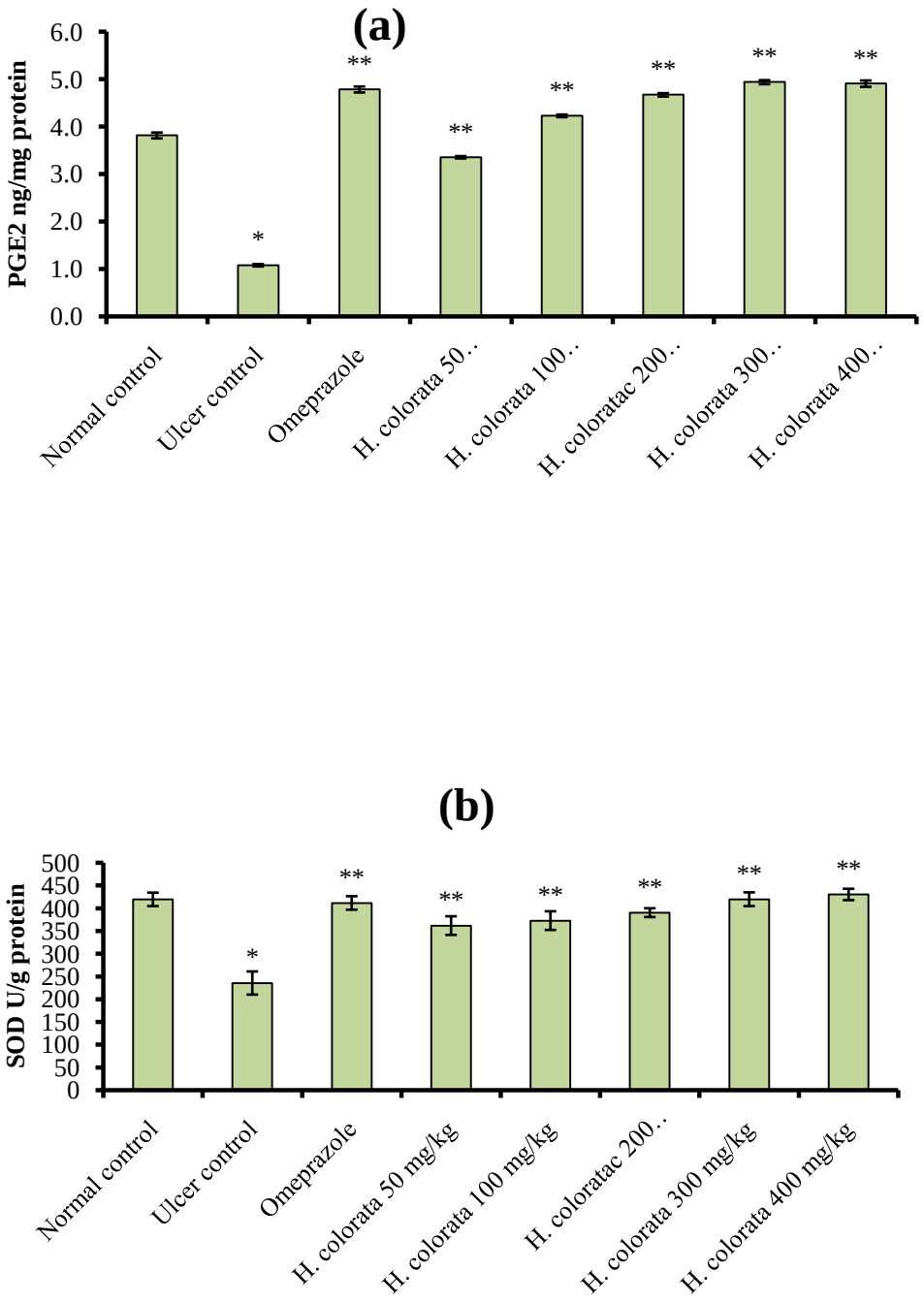


Figure 4.38a, b: Effect of *H. colorata* leaf extract on the level of **(a)** PGE2 and **(b)** SOD in the gastric tissue homogenate collected from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal group. ***P*<0.05 compared to ulcer group.

In the gastric tissue homogenate, both PGE_2 and SOD activities in ulcer control group were significantly lower compared with normal control group (Table4.4). Administration of omeprazole or β-sitosterol before giving the animals HCl/Ethanol significantly increased the level of PGE_2 and SOD in the gastric tissue homogenate much better than Octyl gallate-treated group and compared with ulcer control group. On the other hand, administration of HCl/ethanol significantly increased the MDA level of gastric homogenate in ulcer control group compared with normal control. The level of MDA in β-sitosterol-treated group animals is less than that of Octyl gallate-treated animals although high dose Octyl gallate treatment showed significant increase in the level of PGE2 and SOD and reduced level of MDA than ulcer control group.

Table 4.4: Effect of β-sitosterol and Octyl gallate treatment of the level of GPx, SOD and MDA in the gastric tissue homogenate collected from all animals

Group	PGE2 ng/mg protein	SOD U/mg protein	MDA nmol/mg protein
Normal control	62.00 ± 4.46	356.83 ± 20.84	80.17 ± 1.92
Ulcer control	19.33 ± 2.28**	228.83 ± 23.51**	299.33 ± 21.93**
Omeprazole	47.00 ± 3.07*	330.67 ± 26.39*	141.50 ± 13.27*
B-sitosterol 5 mg/kg	45.17 ± 3.52*	250.33 ± 25.74	212.33 ± 19.78*
B-sitosterol 10 mg/kg	65.17 ± 3.87*	431.67 ± 10.30*	124.50 ± 6.44*
Octyl gallate 5 mg/kg	19.67 ± 1.17	150.67 ± 14.46	263.33 ± 28.30
Octyl gallate 10 mg/kg	52.71 ± 2.69*	330.43 ± 13.47*	150.57 ± 13.63*

Data are presented in the form of Mean ± SEM. ** $P<0.001$ compared to normal control. * $P<0.001$ compared to ulcer control.

4.8.9. The Level of COX-II in the Gastic Tissue Homogenate

The effect of *J. sambac* and *H. colorata* on the level of COX-II in the gastric tissue homogenate collected from all rats is indicated in Figures 4.39 and 4.40. Both plant extracts revealed high level compared to ulcer control which showed significantly low level compared to normal control group rats. Adminstration of *J. sambac* extract increased the level of COX-II from 0.40 ± 0.03 in the ulcer group to 5.27 ± 0.61 ng/ mg protein in the high dose-treated group, while in *H. colorata*, the level improved from 0.59 ± 0.18 in the ulcer group to reach 6.39 ± 0.31 ng/mg protein in the high dose-treated rats.

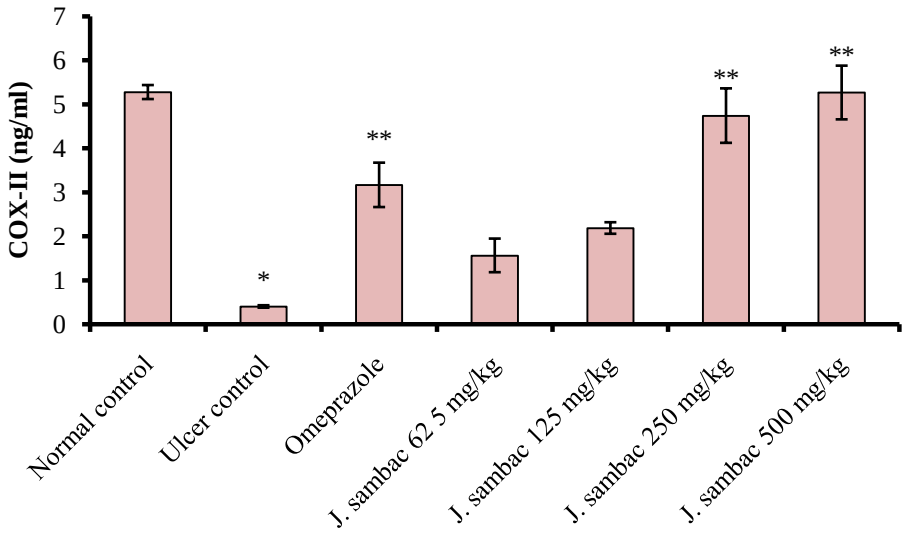


Figure 4.39: Effect of *J. sambac* leaf extract on the level of gastric level of COX-II in the gastric tissue homogenate collected from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal control. ***P*<0.05 compared to ulcer control.

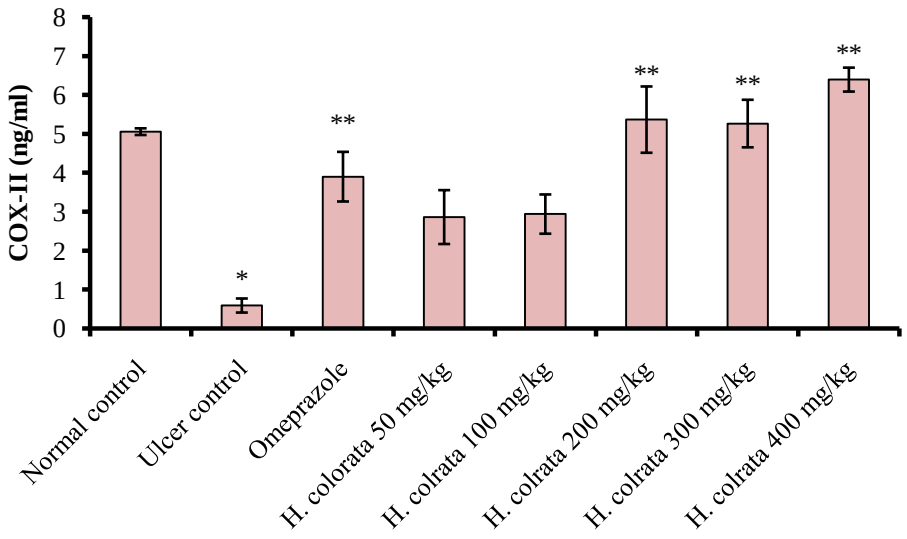


Figure 4.40: Effect of *H colorata* leaf extract on the level of gastric level of COX-II in the gastric tissue homogenate collected from the rats of all the experimental groups. Data are presented in the form of Mean ± SEM. **P*<0.05 compared to normal control. ***P*<0.05 compared to ulcer control.

4.9. Isolation of β-Sitosterol from *J. sambac* and Octyl Gallate from *H. colorata* crude extracts

4.9.1. Isolation of β-sitosterol from the Pertroleum Ether extract of *J. sambac* (JS) leaves

Column chromatography of the crude extract (CC) resulted in 14 fraction as shown in (Figure 4.41 and Table4.3) Thin layer chromatography of the combined fractions JS-1-5, JS-6-8 and JS-9-10 and JS-11-14 showed that fraction 6-8 (JS-6-8) contained β-sitosterol (*Rf* 0.36) (Figure 4.41). When fraction JS-6-8 was re-purified by column chromatography, 16 sub-fractions were obtained. Screening the sub-frations by TLC versus β-sitosterol standard showed that sub-fraction JS-6-8-11 contained the target compound β-sitosterol (Figure 4.42) as detected by HPLC profiling after 3 repeated times at Rt 23.6 (Figure 4.43). The mass of the purified compound was determined by LCMS as m/z 415.3023 as shown in Figure 4.44.

Table4.3: Data of fractions isolated from CC1; Fractionation of *Jasminum sambac* (JS)

Fration no	Solvent elution (%H:% A)	Weight test tube + sample (g)	Weight test tubes (g)	Weight of samples (g)
JS1-5	JS 1 (100H: 0A) JS 2 (100H: 0A) JS 3 (100H: 0A) JS 4 (90H: 10A) JS 5 (90H: 10A	5.1473	5.1459	0.0014
JS6-8	JS 6 (90H: 10A) JS 7 (80H: 20A) JS 8 (80H: 20A)	5.6651	5.1551	0.5100
JS9-10	JS 9 (80H: 20A) JS 10 (80H: 20A)	5.2131	5.1770	0.0361
JS-11-14	JS 11 (80H: 20A) JS 12 (60H: 40A) JS 13 (50H: 50A) JS 14 (0H: 100A)	5.1683	5.1602	0.0081)

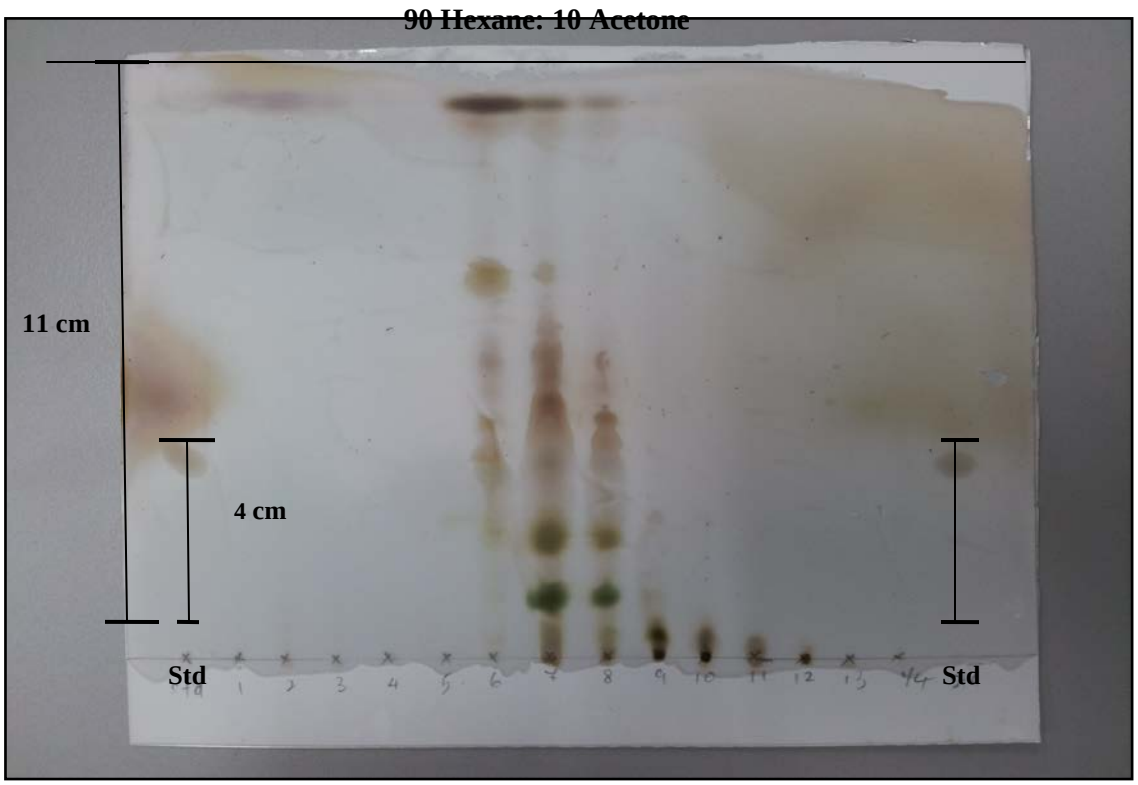


Figure 4.41: TLC profiling of 14 fractions versus β-sitosterol standard (Std) using 90 Hexane: 10 Acetone

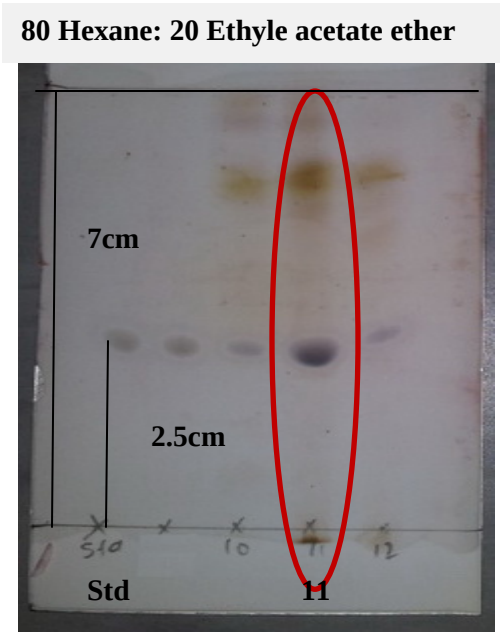


Figure 4.42: TLC profiling of subfractions JS-6-8-10, JS-6-8-11 and JS-6-8-12 using 90 Hexane: 10 Acetone. The red circle indicates subfraction JS-6-8-11 that contains β-sitosterol compound

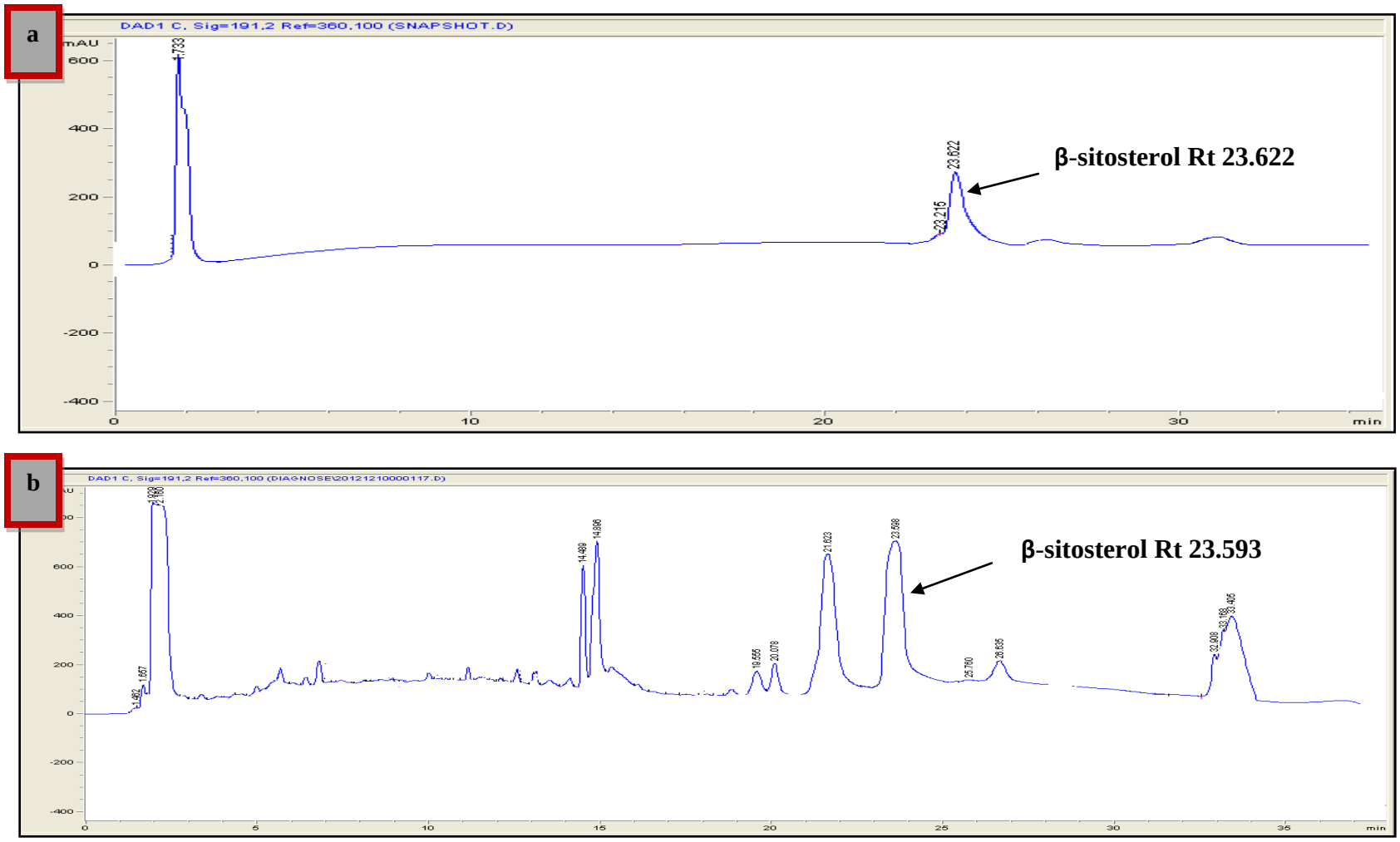


Figure 4.43: HPLC profiling of **(a)** β-sitosterol standard **(b)** subfraction JS-6-8-11 showing β-sitosterol

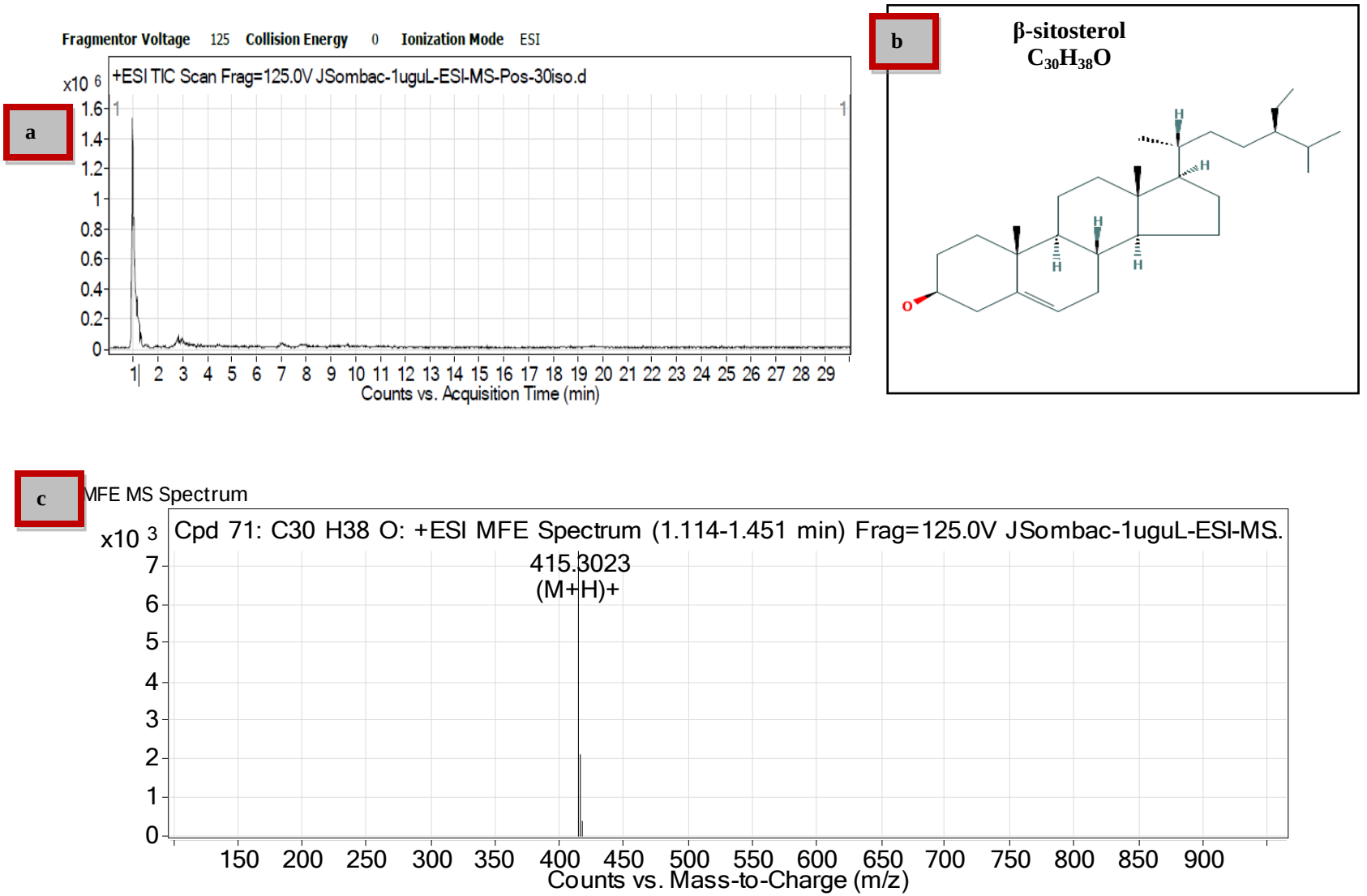


Figure 4.44: **(a)** LCMS chromatogram of sub-fraction JS-6-8-11. **(b)** Structure of β-sitosterol **(c)** MS of β-sitosterol isolated from *J. sambac* crude extract

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4.9.2.Isolation of Octyl gallate from Petroleum Ether *H. colorata* (HC) crude extract

Column chromatography of the ethanol extract of *H. colorata* produced 4 fractions which when screened by TLC versus Octyl gallate standard, the target compound was detected in fraction 4 (R_f = 0.2) as shown in Figure 4.45. HPLC profiling of fraction 4 versus octyl gallate standard confirms the results (Rt 6.484) (Figure 4.46). The mass of the isolated octyl gallate was determined by LCMS as m/z 283.1551 as shown in Figure 4.41.

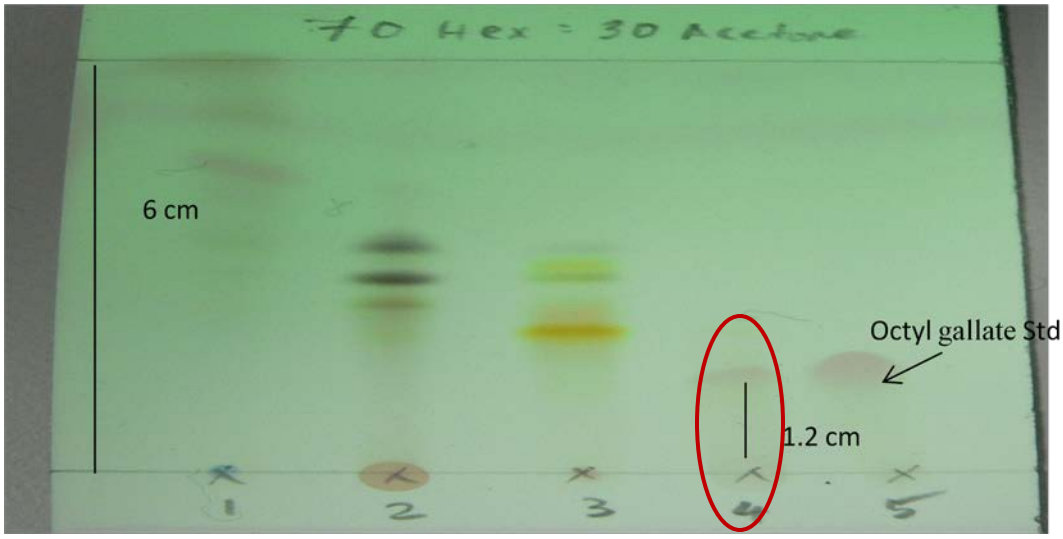


Figure 4.45: TLC profile of 4 fractions versus Octyl gallate standard. The red circle represents fraction HC-4 that contains Octyl gallate compound as compared to the standard.

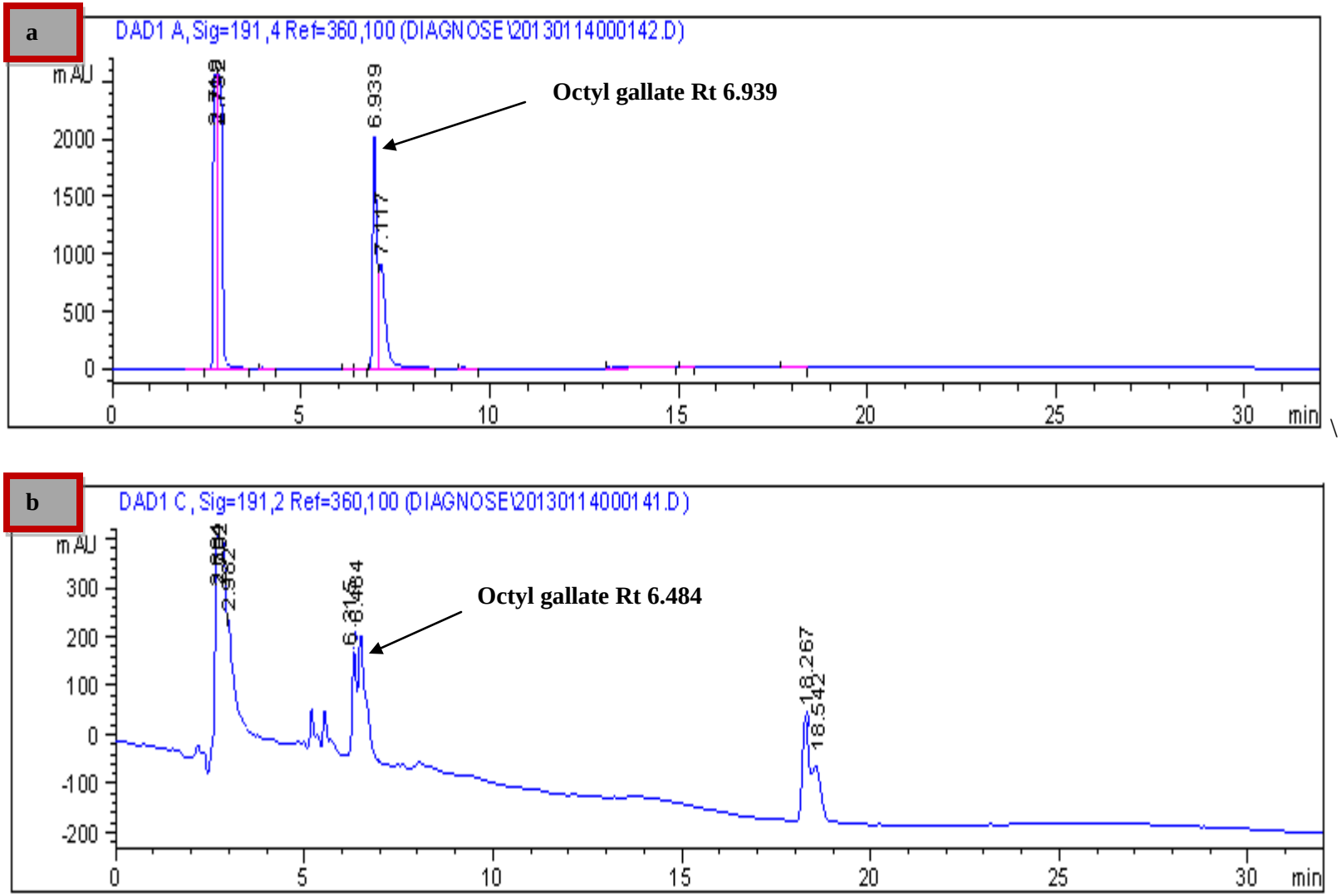


Figure 4.46: HPLC profiling of **(a)** Octyl gallate standard **(b)** Fraction HC-4 showing Octylgallate

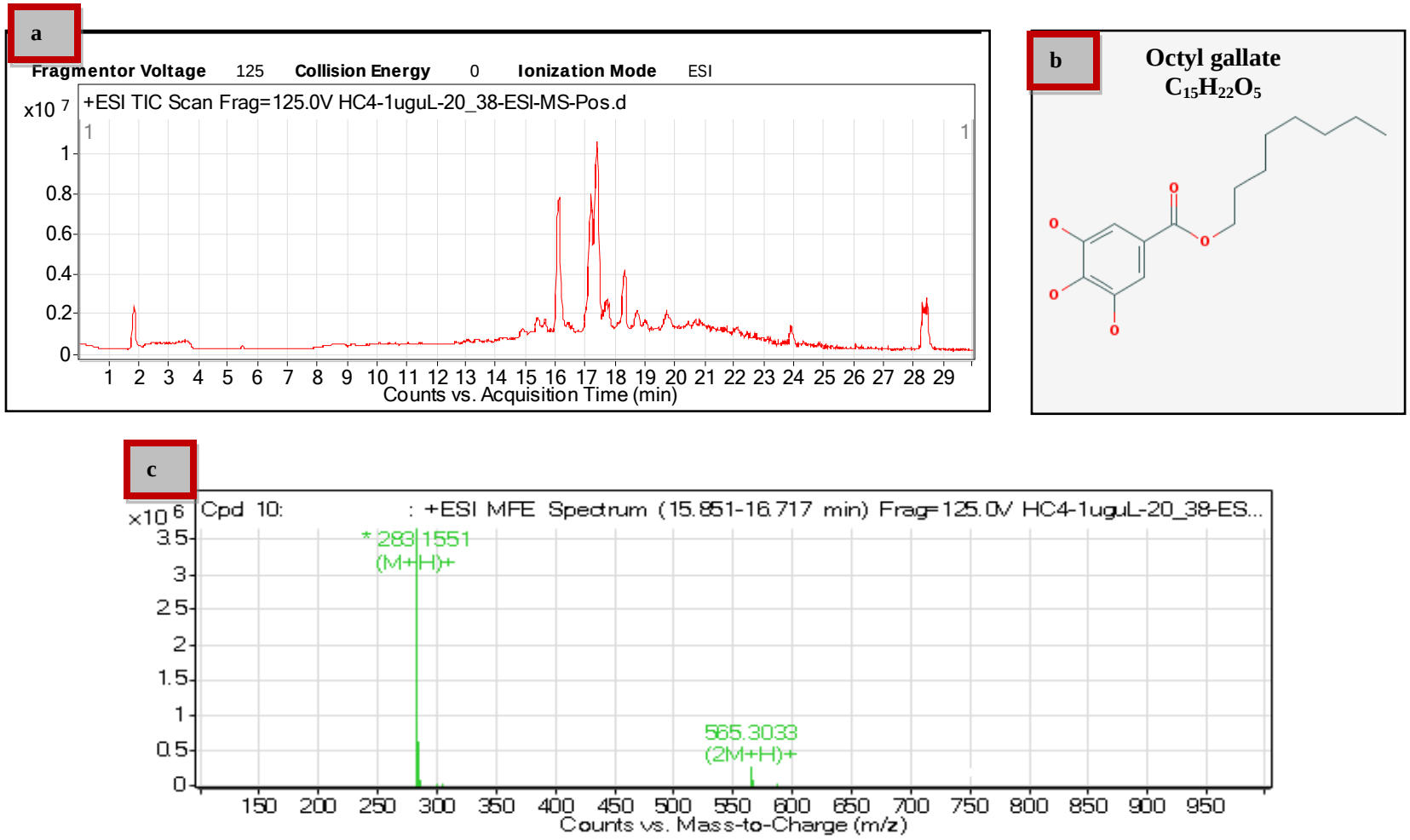
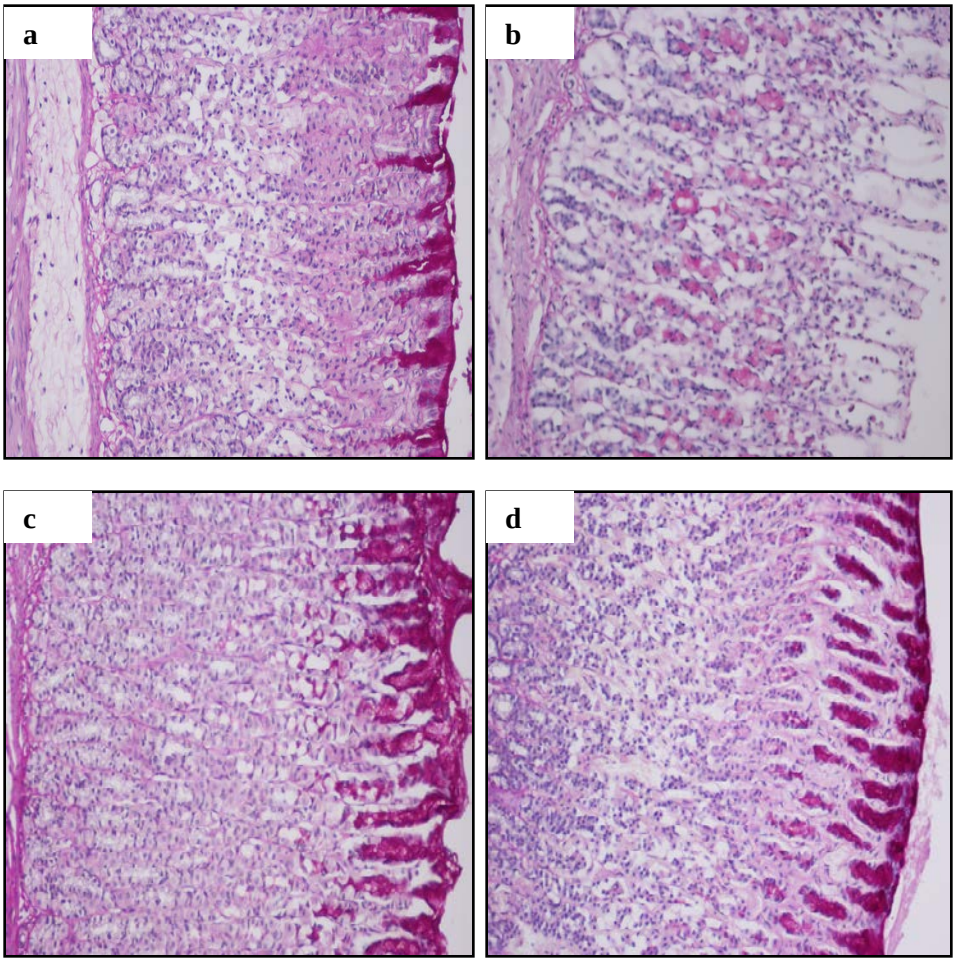


Figure 4.47: **(a)** LCMS chromatogram of fraction HC-4 **(b)** Structure of Octyl gallate **(c)** MS of Octly gallate isolated from *H. colorata* crude extract

4.10.Gastroprotective Activity of β-Sitosterol and Octyl gallate Compounds against HCl/Ethanol Induced Gastric Damage in Rats

4.10.7.Periodic acid Schiff (PAS) of mucosal glycoproteins

Figure 4.48 shows increase in the PAS staining of the gastric mucosa of rats pre-treated with *β*-sitosterol and Octly gallate with better staining in the former compared with ulcer control group indicating increase in the glycoprotein content of gastric mucosa. These results indicate that β-sitosterol-treated group reversed the decrease in PAS staining induced by HCl/ethanol and protected the gastric mucosa from the acidified ethanol better than Octyl gallate.



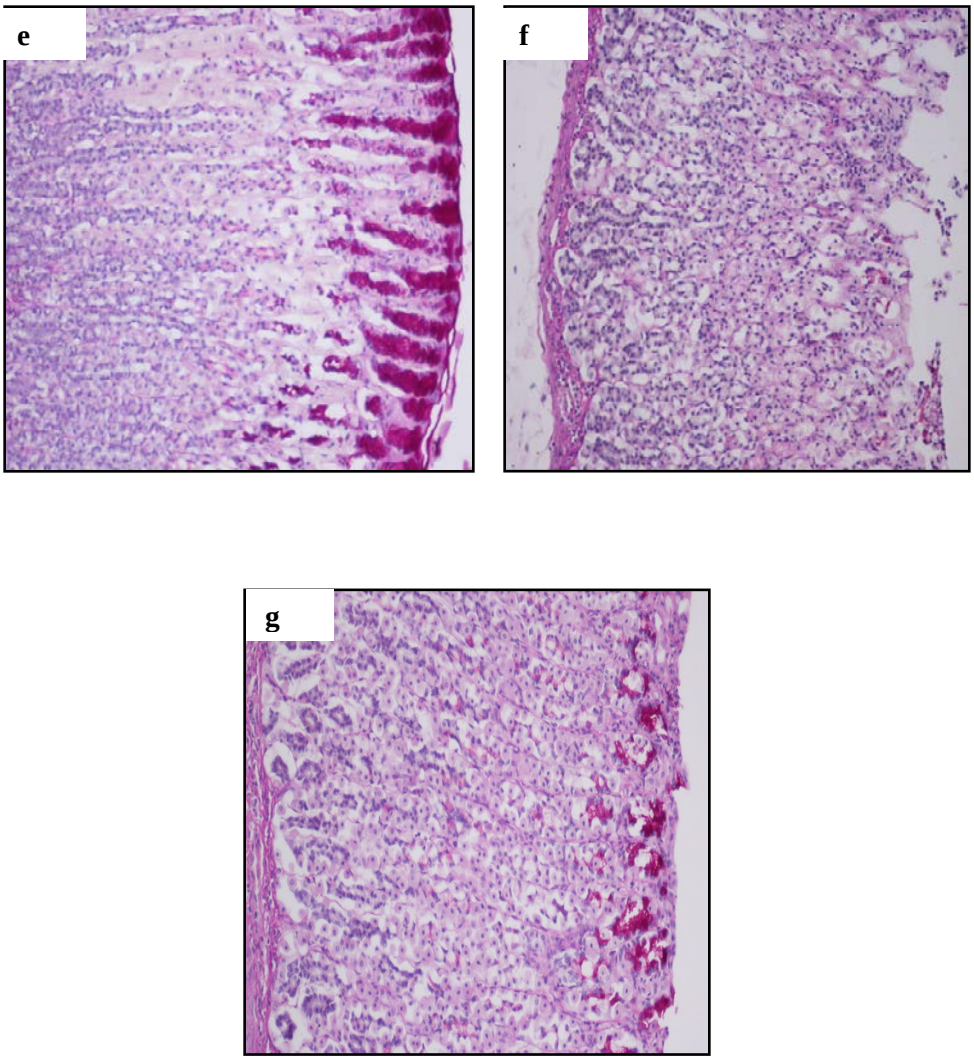


Figure 4.48: Effect of β-sitosterol and octyl gallate on glycoprotein of the gastric tissue-PAS staining in HCl/ethanol-induced gastric ulcer in rats. (a) Rats treated with 5 ml/kg of CMC showed no effect on secretion of mucus. (b).Ulcer control showed inhibition in the secretion of the gastric mucosa. (c) Rat treated with omeprazole (20 mg/kg) showed increased secretion of mucus. (d) Rat treated with β-sitosterol (5 mg/kg) showed high mucus secretion. (d) Rat treated with β-sitosterol (10 mg/kg) showed high mucus secretion (e) Rat treated with Octyl gallate (5 mg/kg) showed no Pas stain indicating no mucus secretion (g) Rat treated with Octyl gallate (10 mg/kg) showed mild Pas stain with mild secretion of mucus (PAS stain 20x).

CHAPTER V

DISCUSSION AND CONCLUSION

Both sexes of rats were showed no sign of toxicological effect or mortality during the experimental period after a single oral dose administration of the extract at 2 and 5 g/kg as compare to control group.

Generally, antioxidant substances in fruit and vegetable play an important role as a health protecting aspect. Scientific research proves that antioxidants are able to decrease the risk of chronic diseases including cancer, heart disease and peptic ulcer. The primary sources of plant antioxidants are vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens that have been established as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties(Kadhum et al., 2011).

Different antioxidant activity assays have been utilized to detect the antioxidant activity of foods. In this study, DPPH radical scavenging activity assay as well as their relations with total phenolic compounds has been performed to monitor the antioxidant activity *of J.sambac and H.colorata.* On the other hand, *H. colorata* crude extract showed higher IC50 > 50 µg/mL indicating that the DPPH scavenging power of *J.sambac* extract is higher than that of *H. colorata* extract. The total phenolic content of *J. sambac* crude extract showed higher value (65.70 ± 0.25 mg GA/mg extract) compared to less value in *H. colorata* (45.70 ± 0.22 mg GA/mg extract).

The results indicated that *J.sambac* exhibited high antioxidant activity in comparison with other plant extract.

Ethanolic plant extracts showed a higher scavenging percentage than the aqueous plant extracts.The results are agreed with previous study which was evaluated the antioxidant activity of some Malaysian medicinal plants by using different antioxidant assay involving DPPH. It was predicted that the highest antioxidant activity was found in the plants extracted with ethanol solvent(Sultana et al., 2009). Another study was showed that the type of solvent significantly influenced the measurement of antioxidant properties of plant extract(Lapornik et al., 2005).

Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages. Several studies have been used this to measure phenolic contents in fruits and vegetables(Dudonné et al., 2009). The total phenolic content of each plant were determined based on the standard curve (Appendix 2) and detected phenolic compounds presented in mg/g in plant extracts equivalent to gallic acid.

It is important to note that throughout the period of wound treatment, the *J.sambac and H.colorata* extract did not cause irritation or pain to the animals as the rats neither show any signs of restlessness nor scratching/biting of wound site when the extract were applied. All the surgical were carried out under sterile conditions and animals were intenstively observed for any infection those which showed signs of infection were separated and removed from the study. This is very important and researchers proved that the control microbial infection is necessary for better healing and its management(Nayak et al., 2006).

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation,

epithetlialization, and wound contraction resulting in a smaller amount of apparent scar tissue(Midwood et al., 2004). In this study, topical application of *H. colorata and J.sambac* extract to excision skin wound in rat demonstrated improved rate of injury contraction as well as extreme decrease in healing time than control, which could be due to increased epitheliazation. The *H. colorata*-treated wound following five days displayed noticeable aridness of injury limits with tissue renewal compared to *J.sambac* in low dose.

However, histological evaluation of wound area of treated groups confirmed the results by increased cellular infiltration, angiogenesis, increased fibroblast and collagen deposition, and the underlying mechanisms of topical *H. colorata and J.sambac* action. This may possibly be due to chemotactic outcome increased by plant concentrate attracting the inflammatory cells in the direction of the wound site. Enhanced cellular rise can be due to the mitogenic action of the plant concentrate, which could have considerably added to healing course of action. Also *H. colorata*-and *J.sambac* treated groups led to considerably lesser injury region on thenth day of post-wound different from placebo rats. The amount of protein achieved from the treated groups of both plants considerably enhanced when contrasted with placebo rats. The amount of proliferating protein in granulate tissue reflected the speed of cellular increase, and accelerated the synthesis and movement of fresh cells into the wound area. Outcomes of Masson's trichrome staining of cross-sections at Day-10 injuries were as well in harmony with these findings. Increased penetration of cells at the injury site from rats treated with plants concentration. These cells played a significant task in matrix deposition and the resolution of tissue reliability as shown by collagen content at the wound sites. Collagen is a main cause of enhanced tensile strength of the healing wound (Bhartiya et al., 1992).

Administration of *H. colorata and J.sambac* exerted useful results on injury curing by stirring the deposition of collagen and angiogenesis. Angiogenesis increased the release of oxygen as well as additional nutrients that were essential for local collagen synthesis (Buemi et al., 2004). Cutaneous injury repair goes parallel with a structured and describable sequence of biological actions beginning with injury closure as well as making progress toward the repair and amendment of injured tissue (Phillips et al., 1991).It is known that Reactive Oxygen Species (ROS) are harmful to injury curing course of action owing to the destructive consequences on cells and tissues. Absorbable synthetic biomaterials are deemed to be tainted via ROS (Aliyev et al., 2004). Free radical-scavenging enzymes are a cytoprotective enzymatic group that has a crucial role in the decrease, de-activation as well as elimination of ROS and controlling injury healing course of action. Healing wounds form granulation tissue which plays the role of filling the dermal defect as well as providing a structural framework for the deposition of newly synthesized collagen. The arrival of macrophages and large number of mesenchymal cells and capillaries that make up the granulation tissue may as well supply substrates as well as inducers for re-epithelialization of the injury (Buckley et al., 1985). A postulation states that applying agents that cause fibroblast and/or endothelial cell increase to healing-impaired wounds could enhance the speed and extent of granulation tissue development and arouse wound repair (Grotendorst et al., 1985). In reaction to tissue loss, fibroblasts multiply and migrate into the wound area until the wound is occupied by fibroblasts and extracellular matrix (Clark, 1993). Cellular reduction is more significant earlier on than collagen reduction in decreasing the diameter of the wound (Ehrlich & Rajaratnam, 1990).

In this study, homogenate tissue obtained from the wound area were dressed with *H. colorata* and *J.sambac* demonstrated considerable reduction in the level of MDA as well as

uplifting the levels of antioxidants enzymes in reaction to oxidative nervous tension. Free radicals and reactive oxygen species (ROS) are constantly formed in human body. These oxygen species are the source of cell injury. Consequently, tissues should be sheltered from oxidative injury through intracellular and extracellular antioxidants (Halliwell, 2006). SOD converts superoxide to hydrogen peroxide which is subsequently changed into water by catalase in lysosomes or by GPx in mitochondria (Johansen et al., 2005). Abridged actions of SOD as well as GPx in tissue injury homogenate in placebo control rats have been examined in our study. The reduction in actions of SOD along with GPx in tissue homogenate may possibly enhanced manufacture of reactive oxygen radicals that can themselves decrease the action of these enzymes (U.N. Tripathi & D. Chandra, 2009). Current application of *H. colorata* and *J.sambac* reinstates the actions of these enzymes and may assist to evade the harmful outcomes of free radicals.

Peptic ulcers are caused by an imbalance between the protective and the aggressive mechanisms of the mucosa, and are the result of the association of several endogenous factors and aggressive exogenous factors that are related to living conditions(Moraes de Carvalho et al., 2010).In the HCl/ethanol-induced gastriculceration model, HCl causes severe damage to gastric Mucosa(AlRashdi et al., 2012), whereas ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors like the secretion of bicarbonate and production of mucus(AlRashdi et al., 2012). Ethanol-induced gastric lesions impaired gastric defensive factors such as mucus and mucosa circulation(Rony et al., 2011). Ethanol causes necrotic lesions of the gastric mucosa in a multifactorial way. It can reach the mucosa by disruption of the mucus-bicarbonate barrier and cause cell rupture in the wall of blood vessels. These effects are probably due to biological actions, such as of lipid peroxidation, formation of free radicals, intracellular

oxidative stress, changes in permeability and depolarization of the mitochondrial membrane prior to cell death. Oral administration plant extract could be attributed to its anti-inflammatory activity as proved previously(Rony et al., 2011). This anti-inflammatory activity could also be a key factor in the prevention of gastric ulcer as reported by Swarnakar et al. Excessive production of myeloperoxidase (MPO) that exists in neutrophil leukocyte cells and catalyses the formation of toxic hypochlorous acid (HOCl) from hydrogen peroxide causes cell membrane damage by lipid peroxidation. MDA is the final product of lipid peroxidation and is used to determine lipid peroxidation levels in tissues (Dursun et al., 2009). Gastric MDAs were increased by ulcer control group and decreased by *J. sambac and H.colorata* extracts administration, another indicator of a possible antioxidant activity of this plant. Studies have shown that the excessive recruitment and metabolic activation of neutrophils generate free radicals in several models of gastric damage resulting in inflammation dependent tissue damage(Bandyopadhyay et al., 2001). In this study, we notice that the mucosal folds surface was flattening this may be the effect of JS and HC leaves extract might be due to a decrease in gastric motility. It is reported that the changes in the gastric motility may play a role in the development and Prevention of experimental gastric lesions (Bandyopadhyay et al., 2001). Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotizing agents and reduce the volume of the gastric irritants on rugal crest (Abdulla et al., 2010). Ethanol produces a marked contraction of the circular muscles of rat fundic strip. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress, at the crests of mucosal folds leading to necrosis and ulceration Ethanol produces a marked contraction of the circular muscles of rat fundic strip. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress, at the crests of mucosal folds leading to necrosis and ulceration(Mahmood et al.,

2010). Gastric tissue homogenate from animals pretreated with omeprazole or plant extract showed significant antioxidant activity by decreasing the levels of MDA and by elevating the levels of PGE2 and SOD in response to oxidative stress due to absolute ethanol administration. Free radicals and reactive oxygen species (ROS) that are continuously produced in human body are the cause of cell damage. Therefore, tissues must be protected from oxidative injury through intracellular as well as extracellular antioxidants(Ajith & Janardhanan, 2007). SOD converts superoxide to hydrogen peroxide (H2O2) which is then transformed into water by catalase in lysosomes or by glutathione peroxidase (GPx) in mitochondria (Uma Nath Tripathi & Deepak Chandra, 2009). SOD-mediated catalysis of superoxide radical anion (O2•−) into less noxious hydrogen peroxide (H2O2) represents the first line of antioxidant defense. In our study, SOD activities were significantly reduced after ethanol administration in ulcer control group, and this reduction was prevented by pretreatment with *J. sambac and H.colorata* extracts. Reduced activities of SOD in gastric tissue homogenate in ulcer control group that have been observed in our study may be due to increased production of reactive oxygen radicals that can themselves reduce the activity of these enzymes(Ajith & Janardhanan, 2007). The reduction of these enzymes in gastric tissue homogenate may lead to a number of deleterious effects. Any compound, natural or synthetic with antioxidant activities might contribute towards the total/partial alleviation of such damage. Lipid peroxidation was found to be an important pathophysiological event in a variety of diseases including gastric ulcer(Ajith & Janardhanan, 2007). It is well known that MDA from lipid peroxidation reacts with DNA bases and induces mutagenic lesions(Marnett, 2000). Pratibha et al. showed that the activated oxygen species can in turn induce cellular events such as enzyme inactivation, DNA strands cleavage and also membrane lipid peroxidation. Both plants play a protective role against gastric ulcer. Its antiulcer effect is related to increasing secretion of adherent mucus and pH of gastric

content, which may inhibit generation of oxygen-derived free radicals, and decrease the consumption of SOD and maintain content of MDA at normal level. Prostaglandin E2 (PGE2) plays an important role in the regulation of gastric mucus secretion. PGE2 has protective effects against various gastric injurymodels (AlRashdi et al., 2012; Luiz-Ferreira et al., 2010). Ethanol has been shown to reduce the mucosal PGE2 content (Luiz-Ferreira et al., 2010). PGE2 is the most abundant gastrointestinal prostaglandin and it regulates functions of the gut, including motility and secretion (AlRashdi et al., 2012). PGE2 has also been shown to exert a protective action on the stomach through the activation of EP receptors (Dey et al., 2006). The role of PGE2 and COX-2 in mediating the gastroprotective effect of *J. sambac and H.colorata* was investigated. The results of the present study suggest that the gastroprotective effect of plant extracts mediated partially by PGE2 as direct measurement of its mucosal level confirmed that its biosynthesis was significantly enhanced by compound. It has been shown that prostaglandins influence virtually every component of the mucosal defense: stimulating mucus and bicarbonate secretion, maintaining mucosal blood flow, enhancing the resistance of epithelial cells to injury induced by cytotoxins, and inhibiting leukocyte recruitment(Aggarwal et al., 2006). Additionally, earlier studies show that prostaglandins promoted by COX -2 exert a gastroprotective action against gastric mucosal lesions through maintenance of gastric mucus synthesis and secretion(Lee et al., 2010). Hsp70 proteins defend cells from oxidative stress or heat shock. Ethanol-generated ROS normally act to inhibit the expression of HSP and increase the expression of Bax.

Hsp70 prevents these partially denatured proteins from aggregating and allows them to refold. The overexpression of HSP70 noticed in this study could suggest that the plant extract protected the gastric tissues through the upregulation of Hsp70. HSP70 is a 70 kDa

protein from the HSP family present on mammalian cells. It is the most conserved and abundantly produced protein in response to different forms of stress (AlRashdi et al., 2012), such as heat, toxic agents, infection, and proliferation. These Hsp70 inductions led to inactivation of MAPK in alcohol-induced gastric injuries(Choi et al., 2009b) showed that HSP70 could play important role in gastric mucosal adaptation when the PGE2 level is suppressed by NSAID. Oyake et al.2009 added the data overexpression of Hsp70 confers protection against monochloramine-induced gastric mucosal injury. Several publications that Hsp70 inductions improved both short-term survival 2-fold and long-term survival 5-fold in mice challenged with ethanol and endotoxin in mice(Choi et al., 2009b). Hsp70 inductions protected rats against ethanol induced gastric mucosal damages(AlRashdi et al., 2012) , and Hsp70 inductions led to inactivation of MAPK in alcohol-induced gastric injuries(AlRashdi et al., 2012) all raised the possibility of the intervention of phytoceuticals as novel therapeutics for preventing alcohol associated gastric damages.

In this study, β.sitosterol and octyl gallate from petroleum ether extract of *J.sambac and H.colorata* were assessing for gastrointestinal protective against acidified ethanol in experimental animals.The obtained data showed siginificantly reduction in gastric ulcer ($P<0.05$) with comparison to CMC group (negative group).These results were confirmed after histological examination as shown in the result section. In the CMC negative control group, the gastric mucosa was extensively damaged with edema and leukocyte infiltration of the submucosal layer. The rats pre-treated with showed markedly better reductions in gastric lesion where the pre-treated shows significantly improvement ($P<0.05$) with much more of mucosal where less edema,inflammation cells (leukocytes) and mucosal surface damage was tiny lesion.β.sitosterol activity enhance marked reduction of lesions in gastric mucus to differentiate with octyl gallate. The consequences of this prevention due to

relaxation of gastric muscle this increase to expand mucosal surface which exposured to ethanol and minimized the amount of the ulcerologic agent(Araki et al., 2008). These endogenous substances were selected because they are positively related to the protection of various disease as proved previously(Wallace et al., 2007).β-sitosterol group reversed PAS staining induced with acidified ethanol and protected the gastric mucosa from the acidified ethanol better than Octyl gallate.

Conclusion

The acute toxicity profile of *J.sambac* and *H.colorata* rhizome extract could be considered favorable judging from the absence of adverse clinical manifestations in experimental animals.

The current study revealed that wounds dressed with *J.sambac and H.colorata* extracts, as topical application of wounds could significantly be protected and accelerating wound closer compared to placebo group.Histology, such protection was shown to be dose dependent and ascertained by the increased blood capillaries and reduction of inflammatory cells infiltration of granulation tissue compared to negative control. The efficacy of these plants in speeding up of wound healing in rats may be due to effect on antioxidant enzymes. As wound tissue homogenates, there is increase in antioxidant enzymes (SOD and GPx) and decrease in the MDA level. In granulation tissue, there is increased deposition of collagen and angiogenisis.

J.sambac,H.colorata extracts and their selected active compounds had anti-gastric ulcer activity in a dose dependent manner and produced mucus which is important in gastric defense mechanism. Reduction of ulcer areas in the gastric wall as well as by the reduction or inhibition of edema and leukocytes infiltration of the submucosal layers were shown histologically. Immunohistochemistry staining of HSP70 and Bax proteins showed overexpression of HSP70 protein and downexpression of Bax protein in rats pre-treated. Besides that, also significantly increases the SOD and decreased the level of lipid peroxidation (MDA) in gastric tissue homogenates.Finally,obtained data provide essential data of *J.sambac and H.colorata* that can used in pharmaceutical industries and phytochemical chemist might recommend to substitute chemical drugs to prevent mucosal epithelial from acidified ethanol ulcerolegic agent..

Publications

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Ahmed S. Al Rashdi, Suzy M. Salama, Salmah Ismail, Salim S. Alkiyumi, Mahmood Ameen Abdulla. **Enhancement of wound healing activity by Hemigraphis colorata in experimental rats. Submitted to Evidence-Based Complementary and Alternative Medicine.** Reference number 280851.v1(IF 4.77)

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