NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

Gastroprotective Effects of *Rheum Rhabarbarum* Extract against Ethanol-Induced Gastric Ulcer Disease: In Vivo Study

MSc. THESIS

Halo Hameed

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M.Sc. THESIS

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DECLARATION

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

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ACKNOWLEDGEMENT

My sincere gratitude is extended to God Almighty, who has enabled me to accomplish great things in my studies despite the fact that it has not always been easy.

I extend my authentic appreciation to my thesis supervisor Assist. Prof. Dr. Eşref Çelik, whose expertise, encouragement, and constructive feedback have been invaluable. Her mentorship has not only enriched my academic experience but has also shaped my growth as a scholar. I am truly grateful for the time and dedication you have invested in my success.

Heartfelt thanks go to my parents, whose boundless love, sacrifices, and unwavering belief in my potential have been the cornerstone of my achievements. Their encouragement has been my driving force, and I am profoundly thankful for your endless support.

I also want to acknowledge my co-supervisor Dr. Ahmed Mahal from Cihan University-Erbil, Iraq. As well as the entire staff at Cihan University, whose commitment to excellence has fostered a conducive learning environment. The collaborative and supportive atmosphere at the university has significantly contributed to my academic development.

In closing, I extend my appreciation to all those who have played a role, no matter how small, in my academic journey. Their contributions have not gone unnoticed, and I am grateful for the collective effort that has shaped my educational experience at Near East University.

ÖZET

Gastroprotective Effects of Rheum Rhabarbarum Extract against Ethanol-Induced Gastric Ulcer Disease: In Vivo Study

Tarih boyunca insanlar tedavi ve tıbbi amaçlar için bitkilere büyük ölçüde güvenmişlerdir. Geleneksel tıbbı uygulayan eski toplumlar, peptik ülser gibi sindirim bozuklukları da dahil olmak üzere çeşitli sağlık sorunlarına çözüm bulmak için sıklıkla bitki bazlı ilaçlar kullanıyordu. Zamanla peptik ülserlere ilişkin anlayış gelişti ve tıbbi prosedürlerde ayarlamalara yol açtı. Ancak omeprazol gibi geleneksel ülser ilaçları, uzun süreli kullanımda potansiyel yan etkilerle ilişkilendiriliyordu. Bu endişe, daha az toksik anti-ülserojenik özelliklere sahip, daha yumuşak ve potansiyel olarak daha güvenli alternatifler olarak algılanan doğal ilaçlara olan ilginin yeniden artmasına neden oldu. Ülserler de dahil olmak üzere mide-bağırsak sorunlarının tedavisinde tarihsel kullanımıyla Rheum rhabarbarum bu çalışmanın odak noktası haline geldi. Bu nedenle çalışmanın amacı, Rheum rhabarbarum ekstraktlarının sıçanlarda mutlak etanolün neden olduğu mide hasarına karşı anti-ülserojenik potansiyelini değerlendirirken aynı zamanda toksisitesini de değerlendirmekti. 30 adet Sprague Dawley sıçanı her grupta 6 sıçan olacak şekilde 5 gruba ayrıldı. 48 saat boyunca yemekten mahrum bırakıldıktan sonra normal kontrolü temsil eden Grup 1'e sadece distile su, negatif veya ülser kontrolü oluşturan Grup 2'ye %0,5 karboksimetilselüloz (CMC), Grup 3'e pozitif kontrolü temsil eden 20 mg/kg ilaç verildi. Omeprazol. Ayrıca Grup 4'e düşük doz Rhabarbarum metanol ekstraktları (250mg/kg) ve son olarak Grup 4'e yüksek doz Rhabarbarum metanol ekstraktları (500mg/kg) uygulandı. Daha sonra tüm gruplara %96 etanol uygulanarak ülserler oluşturuldu ve daha sonra sıçanlar uyuşturuldu, diseke edildi ve mideleri çıkarıldı ve ülser alanı, pH ölçümü ve mukoza ağırlığının yanı sıra ülser inhibisyonunun da belirlendiği histolojik olarak makroskobik olarak incelendi. Ülser kontrol grubuyla karşılaştırıldığında, hem düşük hem de yüksek dozda R. rhabarbarum özütü alan sıçanlarda intragastrik asitte azalma görüldü. Ayrıca, pozitif kontrol (omeprazol) ile karşılaştırıldığında, R. rhabarbarum özütü benzer düzeyde ülser inhibisyonu gösterdi; yüksek doz, düşük doza kıyasla üstün bir inhibitör etki sergiledi. Negatif kontrolle karşılaştırıldığında mide içeriği hem omeprazol hem de R. rhabarbarum ekstraktı gruplarında daha yüksekti. H&E boyası kullanılarak

yapılan mikroskobik inceleme, Rheum rhabarbarum ile tedavi edilen sıçanlarda ülserli alanda bir azalma, mide epitel savunmasında iyileşme ve submukozal tabaka ödeminde ve inflamatuar hücre infiltrasyonunda azalma olduğunu ortaya çıkardı. PAS boyası, deney gruplarinda ülserli grubagore gastric epitclinde daha yüğun boyama gösterdi. HSP 70 protein ekspresyonu ülserli kontrol grubunda azaldı ve hem omeprazol hem de Rheum rhabarbarum gruplarında arttı. Bax protein ekspresyonu ülserli kontrol grubunda arttı ancak omeprazol veya Rheum rhabarbarum ile önceden tedavi edilen gruplarda azaldı. Ayrıca ülser kontrol farelerinden elde edilen mide homojenatları, omeprazol ve R. rhabarbarum ekstraktı ile tedavi edilenlere kıyasla önemli ölçüde daha düşük SOD ve CAT seviyeleri sergiledi. Son olarak, omeprazol ve R. rhabarbarum özütü uygulanan farelerde mide homojenatlarında önemli ölçüde azalmış TNF-a ve IL-6 seviyeleri görülürken, IL-10 seviyesi belirgin şekilde daha yüksekti. Bu sonuclar, *R. rhabarbarum* ekstraktının potansiyel anti-ülserojenik etkilerini ve bunun çeşitli biyokimyasal ve histolojik parametreler üzerindeki etkisini vurgulayarak, mide hasarını hafifletmedeki terapötik potansiyelini ortaya koymaktadır.

Anahtar Kelimeler: *R. rhabarbarum ekstraktı, Etanole Bağl ülser, Ülser İnhibisyonu, Omeprazol, rats, experimental ulcer*

ABSTRACT

Gastroprotective Effects of Rheum Rhabarbarum Extract against Ethanol-Induced Gastric Ulcer Disease: In Vivo Study

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Throughout history, humans have heavily relied on plants for therapeutic and medicinal purposes. Ancient societies, practicing traditional medicine, often employed plant-based remedies to address various health issues, including digestive disorders like peptic ulcers. Over time, the understanding of peptic ulcers evolved, leading to adjustments in medical procedures. However, conventional ulcer medications such as omeprazole were associated with potential side effects with prolonged use. This concern has prompted a renewed interest in natural remedies, perceived as gentler and potentially safer alternatives with less toxic anti-ulcerogenic properties. *Rheum rhabarbarum*, with its historical use in addressing gastrointestinal problems, including ulcers, became the focus of this study. For this reason, the aim of the study was to assess the anti-ulcerogenic potential of *Rheum rhabarbarum* extracts against absolute ethanol-induced gastric damage in rats while also evaluating its toxicity. 30 Sprague Dawley rats were divided into 5 groups with 6 rats in each group. After being deprived from food for 48 hours, Group 1, which represent normal control received only distilled water, Group 2 which constituted negative or ulcer control received 0.5% carboxymethylcellulose (CMC), Group 3 represented the positive control and were administered 20mg/kg of omeprazole. In addition, Group 4 were administered

low dose *Rhabarbarum* methanol extracts (250mg/kg) and finally Group 5 received high dose *Rhabarbarum* methanol extracts (500mg/kg). Ulcers were then induced by administering 96 % ethanol to all groups, and afterward rats were anesthetized, dissected and their stomach were extracted in order to be examined macroscopically, histologically where ulcer area, pH measurement and mucous weight as well as ulcer inhibition were all determined. Compared to the ulcer control group, rats receiving both low and high doses of *R. rhabarbarum* extract exhibited decreased intragastric acidity. Furthermore, in comparison to the positive control (omeprazole), *R. rhabarbarum* extract demonstrated a similar level of ulcer inhibition, with the high dose displaying a superior inhibitory effect compared to the low dose. Gastric content was higher in both omeprazole and *R. rhabarbarum* extract groups when contrasted with the negative control. Microscopic examination using H&E stain revealed a reduction in ulcerated area, improvement in stomach epithelium defense, and reduced submucosal layer edema and inflammatory cell infiltration in rats treated with *Rheum rhabarbarum*. PAS stain indicated a higher staining intensity of glycoprotein in the gastric epithelium of experimental groups compared to the ulcerated control group. HSP 70 protein articulation decreased in the ulcerated control assembly and increased in both omeprazole and *Rheum rhabarbarum* groups. The expression of Bax protein increased in the ailment-bearing control group but decreased in groups pre-treated with omeprazole or *Rheum rhabarbarum*. Furthermore, gastric homogenates from ulcer control rats exhibited significantly lower levels of SOD and CAT in comparison with those on omeprazole therapy and *R. rhabarbarum* extract. Finally, rats administered omeprazole and *R. rhabarbarum* extract displayed significantly reduced levels of TNF-α and IL-6 in gastric homogenates, while the level of IL-10 was markedly higher. This comprehensive evaluation highlights the potential anti-ulcerogenic effects of *R. rhabarbarum* extract and its impact on various biochemical and histological parameters, suggesting its therapeutic potential in mitigating gastric damage.

Keywords: *R. rhabarbarum extract, Ethanol Induced Ulcer, Ulcer Area, pH Measurement, Mucous Weight, Ulcer Inhibition, Omeprazole.*

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List of Schemes

List of symbols

%	Percentage
C°	Degree Celsius
α	Alfa
♂	Male
♀	Female
+	Positive
-	Negative
=	Equal
UA	Urinalysis

List of abbreviation

mg/Kg	Microgram per kilogram
ml	Milliliter
µm	Micrometer
mm^2	Millimeter Sqaure
g	gram
S.E.M	Standard Error Mean
G	Group
U/ml	Units Per Milliliter
mL/kg	Milliliters per kilogram
nmol/min/mL	nanomoles per minute per milliliter
umol/L	micromoles per liter
Pg/mL	picograms per milliliter
TCM	Traditional Chinese medicine
COX-2	Cyclooxygenase-2
H & E stain	Hematoxylin and eosin stain
PAS stain	Periodic Acid Schiff stain
GU	gastric ulcer
GIT	gastrointestinal tract
e.g.	For example
PPIs	proton pump inhibitors
H. pylori	Helicobacter pylori
PGE2	Prostaglandin E2
ECL cells	Enterochromaffin-like cells
et al	And others
Fig	Figure

HCl	Hydrochloric acid
H+	Hydrogen ion
K+	Potassium ion
$O2^{\bullet -}$	Superoxide
OH^-	Hydroxyl radicals
$HCO3^-$	Bicarbonate
NSAIDs	Nonsteroidal anti-inflammatory drugs
ROS	Reactive oxygen species
SOD	Superoxide dismutase
GSH	Glutathione
H2RAs	H_2 Receptor Antagonists
ER	Estrogen receptors
LPS	Lipopolysaccharide
ER	Endoplasmic reticulum
PBS	Phosphate-buffered saline
CMC	Carboxymethylcellulose
CAT	Catalase
TNF-α	tumor necrosis factor-alpha
IL-6	interleukin-6
IL-10	interleukin-10
MDA	malondialdehyde

Chapter I

Introduction .1

Background 1.1

Human beings have relied on a variety of plants for their therapeutic advantages and medicinal qualities throughout the history of mankind. Ancient societies used traditional medicine, which frequently included using plant-based treatments to treat a variety of health problems, including digestive disorders and what we now know as herbal medicine was founded on the knowledge of these plants and their effects that was passed down through the generations, leading to the creation of a complex tapestry of herbal traditions from various global cultures (Pan, et al., 2014). One of these disorders is peptic ulcers, a painful sores which develop on the inside lining of the stomach, lower esophagus, or small intestine. The earliest known civilizations in human history have a connection between peptic ulcers and the usage of plants in various forms of medicine and therapeutic techniques. This historical trip not only demonstrates the persistence of human curiosity and the will to relieve pain, but it also emphasizes the important contribution that plant-based medicines have made to our comprehension of this illness (Banić, et al., 2011). The Egyptians, Greeks, and Chinese were among the ancient societies who recorded ulcer symptoms and made an effort to create remedies. The first references to plant-based treatment and peptic ulcers can be found in their ancient medical literature and herbal traditions (Oumeish, 1998). For instance, the ancient Egyptians who were known for their contributions to pharmacology and medicine were also among the first to identify the signs and symptoms of peptic ulcers. Papyrus scroll hieroglyphs document their usage of plant-based remedies to relieve gastrointestinal aches and pains. Plants like aloe Vera, which has calming and anti-inflammatory qualities, were often included in their treatments. The Egyptians were aware of the plants' therapeutic properties and the comfort they may provide to those with peptic ulcers (Aboelsoud, 2010). In addition, herbal medicine and the use of herbs to treat gastrointestinal disorders have long been used by the Chinese also. A disorder known as "stomach fire," which resembles the symptoms of peptic ulcers, is recognized by traditional Chinese medicine (TCM). Herbal medicines were used in TCM to balance the body's energy and lessen stomach lining inflammation. Plants like marshmallow root and Chinese skullcap were utilized to treat and ease

stomach pain (Liu, et al., 2015). However, although the knowledge of peptic ulcers changed with time, and medical procedures adjusted to reflect these developments. Yet, with the development of modern medicine, the usage of botanicals to treat peptic ulcers did not disappear. Rather, it saw a comeback within the complementary and alternative medical field. Plant-based treatments for digestive disorders were still used in herbalism, naturopathy, and traditional medical systems where these treatments were frequently combined with mainstream medical procedures (Sadiku & Musa, 2022). Furthermore, in the twenty-first century, research on plants and their possible effect on peptic ulcers has adopted a more methodical, evidence-based approach. To learn more about how plant chemicals may aid in ulcer healing, researchers have dug into their molecular mechanisms. Despite the fact that antibiotics are currently the major treatment for peptic ulcers since they fight Helicobacter pylori infections. Still, there is interest in the possibility of plant-based adjunct medicines (Efferth & Koch, 2011). Rheum rhabarbarum, or rhubarb, which is indigenous to China, Mongolia, and Tibet, among other Asian nations, is one such plant that has been associated with this historical tradition since it has a long history of usage in both cooking and medicine. Its origins, which have been used in conventional treatment, were eventually studied by scientists for being a topic of attention in both traditional and modern medicine due to its exceptional gastroprotective qualities. Its distinct chemical profile is the basis for Rheum rhabarbarum's gastroprotective properties. The medicinal potential of rhubarb is attributed to its content of anthraquinones, tannins, and other bioactive substances. These substances may have a variety of functions, such as supporting the development of mucus in the gastrointestinal tract, which serves as a barrier of defense, and possessing anti-inflammatory and antioxidant qualities (Awaad, 2022).

Traditionally, rhubarb has long been used to ease gastrointestinal distress and support gastrointestinal health. Because of its inherent astringency and anti-inflammatory properties, it was making a great option for treating peptic ulcers and gastritis. While anthraquinones may have a slight laxative effect and help with constipation, which frequently coexists with digestive disorders, rhubarb's tannins are thought to help reduce gastric inflammation. Moreover, its ability to inhibit cyclooxygenase-2 (COX-2), an enzyme involved in the inflammatory response in the stomach lining, is one of the main ways it provides gastroprotection. This anti-inflammatory effect can aid in ulcer healing by lessening ulcer severity (Liudvytska, et al., 2023). However, while rhubarb has the potential to be gastroprotective, its use should be regulated because of its

laxative properties and the presence of oxalic acid, which can be hazardous in high doses particularly those whose digestive systems are delicate. The main symptoms of rhubarb toxicity are related to the ingredients presents in the leaves and include nausea, vomiting, diarrhea, stomach pain, and in extreme situations, kidney damage. Therefore, oxalate poisoning can occur from consuming a large amount of rhubarb leaves, especially when they are uncooked. Kidney stones and other health problems may result from the oxalic acid in the leaves' binding to calcium in the body, which forms crystals that can build up in the kidneys and other organs (Slaughter, et al., 2012).

In conclusion, the history of medicine from prehistoric times to the present day illustrates the continuing connection between the usage of plants and peptic ulcers. The supplementary use of plants and their derivatives continues, despite major advances in medical science's understanding of peptic ulcers (Kumar & Navaratnam, 2013). Moreover, plant-based therapies have long been useful in supporting digestive health and well-being, as demonstrated by rhubarb. With its ability to reduce inflammation, boost mucus production, and act as an antioxidant, rheum rhabarbarum represents a potential supplement for treating gastrointestinal disorders ranging from gastritis to peptic ulcers while taking into account its risk for toxicity (Moon, et al., 2006).

1.2 objectives

1. To evaluate the acute toxicity of *Rheum rhabarbarum* through a series of tests.

2. To assess *Rheum rhabarbarum* extracts anti-ulcerogenic potential against absolute ethanol-induced gastric damage in rats macroscopically.

3. To measure and compare the pH values specifically the intragastric acidity of the gastric juice mucin content after administering *Rheum rhabarbarum* methanol extracts in both low and high doses and to explore its correlation with the presence of gastric ulcers.

4. To assess and compare the amount of mucus production in the stomach following the administration of *Rheum rhabarbarum* methanol extracts in both low and high doses, and to investigate its connection with the occurrence of gastric ulcers.

5. To evaluate the average size of ulcers and the extent of ulcer prevention of ethanol-induced ulcers among rats, considering various pre-treatments administered to the rats.

6. To examine the histopathological characteristics of the stomach using the following staining techniques:

    a. Hematoxylin and eosin (H & E) stain.
    b. Periodic Acid Schiff (PAS) stain.
    c. Immunohistochemistry stain.
    d. Heat shock protein 70 (HSP 70) stain
    e. Bax stain.

Chapter II

Literature Review .2

Introduction 2.1

As the world's population continues to age, the likelihood of developing peptic ulcers may rise, given that older individuals tend to be more vulnerable (Greenwald, 2004). Moreover, the demands of modern life, characterized by hectic work schedules, unhealthy eating patterns, increased rates of smoking and alcohol consumption, as well as elevated stress levels, can collectively exert over time detrimental effects on an individual's digestive well-being. One noteworthy consequence is the heightened risk of peptic ulcer formation (Miller, 2010). On the other hand, conventional ulcer medications including omeprazole are thought to have a number of possible side effects when used for a long-term (Al Ali, et al., 2022). Consequently, individuals are drawn to the idea of natural remedies, perceiving them as gentler and potentially safer options for their health with less toxic anti-ulcerogenic principles. Rhubarb for instance has been used traditionally to address gastrointestinal issues such as ulcer (Khattak, Syeda & Shahzad, 2020). Yet, there is continuing scientific research on its safety and effectiveness in preventing ulcers.

Stomach and Gastric Ulcer Formation 2.2

The stomach, a part of the gastrointestinal system, is a muscular, hollow, and expanded section of the digestive tract. It plays a central role in the digestion process since food and other substances acquired from the esophagus are digested in the stomach, before being discharged into the small intestine. In addition, it creates an acidic environment, releasing proteases and hydrochloric acid to protect against bacterial intrusion. The stomach consists of four distinct layers: the innermost layer known as the mucosa, followed by the submucosa, the muscularis externa, which includes inner oblique, middle circular, and outer longitudinal layers, and finally, the outermost layer referred to as the serosa (Mahadevan, 2014).

Although the gastrointestinal system is continually submerged in acid and proteolytic enzymes, yet it has an amazing ability to hold together. A superficial mucosal damage that results from ingesting toxic substances or from direct physical trauma heals quickly. Its prolonged adherent alkaline mucus layers, rapid cellular turnover, efficient mucosal blood flow, and prostaglandin E series, which thicken the gel mucosal layer and encourage bicarbonate ion production, are the reasons behind its membrane protection and process of repair. All of which shield the mucosal lining of the stomach from corrosively acidic secretions and other irritants (Playford & Ghosh, 2005).

On the other hand, despite these various protective mechanisms one in seven complaints are related to gastrointestinal tract (GIT) diseases, which are widespread. An abundance of these are duodenal and stomach disorders where one of the most prevalent digestive system conditions in the current era of globalization is gastric ulcer (GU) that causes agony for millions of people worldwide and has a high morbidity rate of roughly 5–10% during a person's lifetime. Thus, representing a significant public health burden (Lanas & Chan, 2017). The pathogenesis and etiology of gastric ulcer disease (GU) are still up for debate, but a number of studies have shown that the disease is caused by a critical imbalance between the protective factors of the gastric mucosa particularly prostaglandin levels and antioxidant enzyme activity and invasive factors, such as continuous non-steroidal anti-inflammatory consumption. This imbalance disrupts the gastric mucosal defensive barrier, which in turn causes gastric ulcers (Woolf & Rose, 2019). Moreover, it has been extensively documented that ethanol-induced oxidative stress leads to mucosal ulceration in the stomach by producing extremely harmful free radicals with high cytotoxicity (Aziz, et al., 2019).

By definition, a lesion or sore that appears on the skin or on the mucous membranes lining internal organs is called an ulcer. It is any tiny area of skin or internal tissue surface that becomes inflamed and has shallow breaches in it. Even while they might not seem like much, little or shallow breaks could be signs of ulcers. In their early stages, they may resemble a second-degree burn and exhibit blistering, reddening, or both. If treatment is not received, the inflammation causes tissue necrosis, which increases the risk of infection and bleeding from the lesion. Eventually, the lesions gradually get more crater-like as they develop and become open, festering sores recognized as peptic ulcers (Black, et al., 2007).

2.2.1 Mechanism of gastric acid secretion

The use of different medicines to reduce stomach acidity is justified in part by the physiological regulation of acid output by parietal cells. Parietal acid secretion is triggered by three main pathways: (1) Promoting local paracrine activation occurs via histamine secretion from enterochromaffin-like (ECL) cells; (2) neurological excitation through the vagus nerve; and (3) endocrine stimulation through the release of gastrin from antral G cells (Jain, et al., 2007). However, prostaglandin E2 (PGE2), the primary PG produced by cyclooxygenases, continues to be essential for maintaining stomach homeostasis. Since prostaglandin E2 suppresses acid secretion, fluctuations in its levels brought on by NSAID medication continue to be a serious risk to the integrity of the stomach mucosa (Thomson & Shaffer, 2012).

On the other hand, the process of stimulating acid secretion usually starts with an increase in intracellular calcium and/or cAMP levels. This is followed by the activation of a cascade of protein kinases that is dependent on cAMP. This cascade causes the proton pump enzyme, H+-K+-ATPase, to translocate and insert itself into the parietal cells' apical plasma membrane (Yao & Forte, 2003). The proton pump is found in dormant form in cytoplasmic tubulovesicles of the resting parietal cell, most likely due to the poor permeability of these membranes to K+. However, the massive proton gradients connected to gastric hydrochloric acid (HCl) production are caused by the exchange of internal protons with extracellular potassium (K+) in an electro-neutral manner., which is catalyzed by the H+-K+-ATPase (Guyton, 2006).

2.2.2 The mucosal defense system of the stomach

The layers of gastric mucosa serve as a barrier, preventing the stomach mucosal cells from being exposed to a variety of harmful luminal chemicals and irritants from both endogenous and foreign sources (Allen, et al., 1993). On contrary, when the hindrance is weakened or the corrosive challenge intensifies, it can lead to the degradation of the underlying tissue, overwhelming the epithelial layers and causing a lesion or ulcer to form (Playford & Ghosh,

2005). Noting that anything that penetrates the mucosal barrier causes the stomach wall to erode and the underlying tissue to become inflamed, leading to gastric ulceration (Kwiecien, et al., 2002).

Functional, humoral, and neural elements make up the majority of the gastrointestinal mucosa's endogenous gastroprotective components against hostile forces. Humoral factors include prostaglandin, hydrogen carbonate, and nitric oxide, whereas basic secretion of mucus, mucosal microvascular circulation, and motility act as functional factors. These variables are all known to support mucosal defense against harmful luminal chemicals (Repetto & Llesuy, 2002). Additionally, mucosal barrier function has a physiological basis that encompasses multiple elements and mechanisms, including mucus coating of epithelial cells, acid-neutralizing component HCO_3, as well as tight junctions connecting epithelial cells, and a high rate of epithelial cell turnover. They could be thought of as the mucosal protective barriers pre-epithelial, epithelial, and sub-epithelial components as seen in figure 2.2 (Valle, 2022).

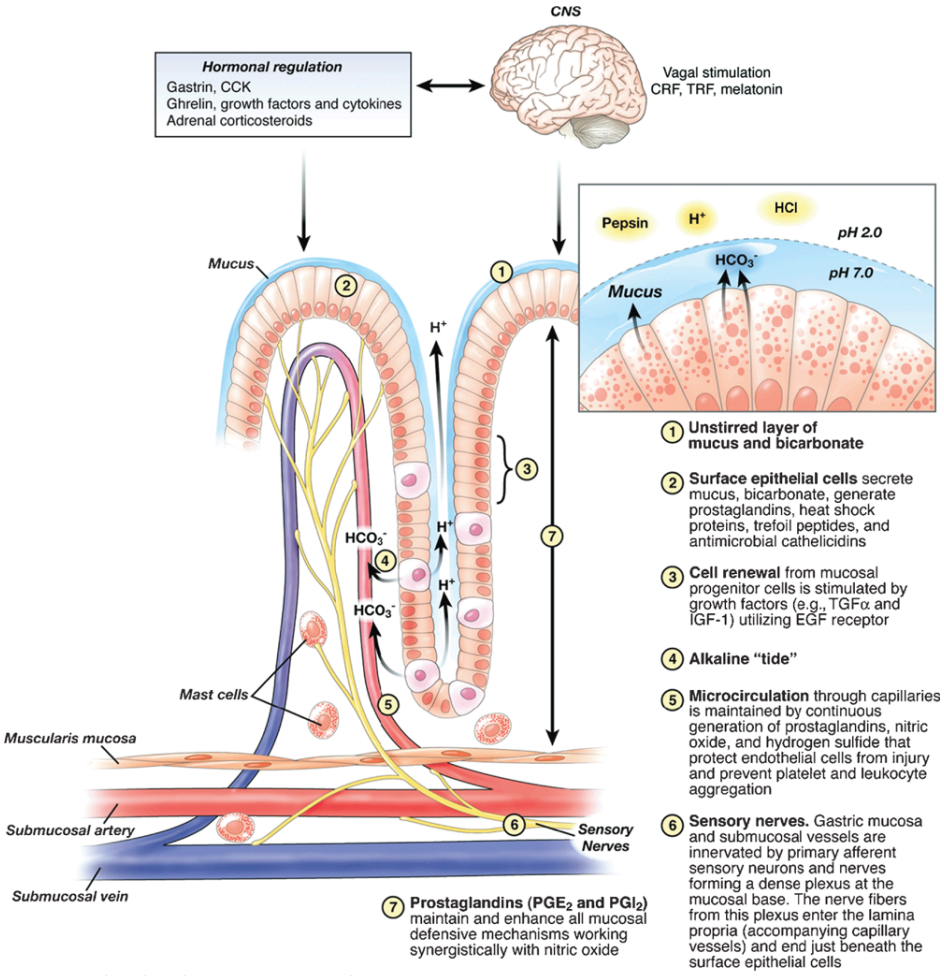


Figure 2.2: *Components that contribute to the protection and healing of the gastroduodenal mucosa (Valle, 2022).*

A mucosal defense layer acts as a chemical-physical fence to several molecules, embracing H+, and is the first line of defense. Mucous gel acts as a non-stirred water barrier that prevents molecules and ions like H+ and pepsin from diffusing. The entire gastrointestinal mucosa is covered in a viscous, elastic, adherent, and translucent gel called gastric mucus, which is made up of 5% glycoproteins and 95% water. Because mucus has the ability to function as an antioxidant, it can lessen the harm that oxygen free radicals do to mucosal tissue (Guha & Kaunitz, 2002). The quantity or the viscous texture of the layers guarding the mucosal outer layer affects the mucus barrier's protective qualities in addition to the gel structure. Intracellular mucus may be discharged into the stomach tissue in response to extracellular oxygen radicals damaging mucus-containing cells. This process scavenges the radicals and averts further damage (Repetto & Llesuy, 2002). As a result, when gastric mucus levels drop, epithelial cells become more vulnerable to damage from substances like aspirin or acid (Kauffman, 1989). Moreover, the

second layer, the surface epithelial cells, produce mucus and bicarbonate as well as form intercellular tight junctions, which together constitute the next line of defense. Numerous growth factors, including basic fibroblast growth factor (FGF), transforming growth factor alpha (TGFα), and epidermal growth factor (EGF), regulate the course of the mucosa's restoration known by way of the process of healing its damaged areas (Guha & Kaunitz, 2002). Lastly, within the stomach submucosal layer lies a complex microvascular system known as the sub-epithelial defense/repair system. Another crucial element of the operation of the gastroduodenal barrier is mucosal blood flow. Luminal acid in the stomach promotes the mucosal microcirculation's transport of vascular bicarbonate into the upper mucous layer, neutralizing H+ that invades from the lumen. The submucosal circulatory bed eliminates harmful metabolic byproducts and supplies O2, HCO_3-, and micronutrients. Endogenous prostaglandins (PGs) are crucial for maintaining mucosal integrity because they facilitate the continual release of HCO_3- and the generation of mucus in the duodenum and stomach (Valle, 2022).

Therefore, as previously mentioned, the development of gastric ulcers is caused by a number of factors, such as an imbalance between the stomach's aggressive (gastric acid and pepsin) and protective factors (mucus, prostaglandins, mucosal blood flow, and mucosal epithelial regeneration). Moreover, factors that raise the risk of ulcers include an uncontrolled use of non-steroidal anti-inflammatory drugs (NSAIDs), Helicobacter pylori infection, improper lifestyle choices like smoking, stress, eating poorly, and drinking alcohol (Nugroho, et al., 2016).

2.3 Types of Gastric Ulcers

Duodenal and stomach ulcers are the two primary forms of peptic ulcers. They differ in some ways even though they have the same causes and the same methods of diagnosis and treatment. Dyspepsia and pain or discomfort in the pit of the stomach can result from either. Utilizing NSAIDs increases the risk of gastric ulcers compared to duodenal ulcers (Rao, et al., 2006).

The lesser curvature, close to the intersection of the antral mucosa and acid-producing parietal cells, is where gastric ulcers are most frequently discovered. This region extends to a region 2-3 cm above the pylorus. The duodenal bulb or the pyloric channel region are typically the sites of duodenal ulcers (Pradeep, 2014). The most typical locations of peptic ulcers in the gastroduodenal mucosa are depicted in Figure 2.3 (Jain, et al., 2007). On the other hand, a high

number of gastric lesions and a considerable proportion of ulcers present in the duodenum are not associated with elevated gastric juices production. Gastric ulcers are characterized by a weakening of the protective mechanisms of the stomach lining, which can result in damage despite low acid secretion. However, gastric ulcers have been categorized into four types (Gear, et al., 1971):

- Type I: which occurs along the lesser curve of the stomach.
- Type II: which is associated with old or coexisting duodenal ulcers.
- Type III: located in the anterior part of the stomach near the pylorus region.
- Type IV: situated near the heart region.

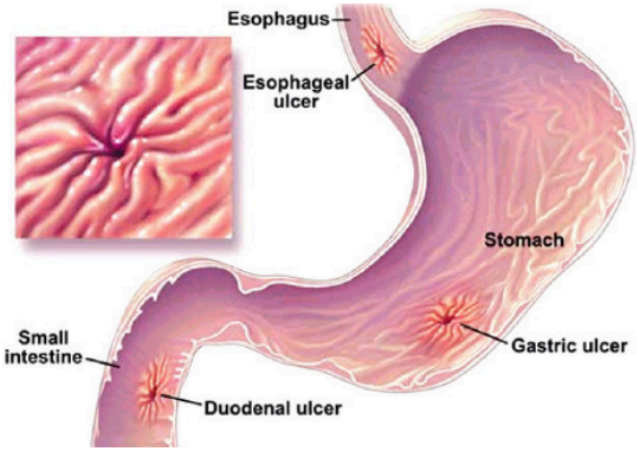


Figure 2.3: *Typical peptic ulcer sites (Jain, et al., 2007).*

2.4 Complications of Peptic Ulcers

Most ulcers typically heal with no sign of complications, yet if an ulcer remains unaddressed or doesn't get well properly, it can result in severe issues. These problems can arise suddenly, particularly in individuals using nonsteroidal anti-inflammatory drugs (NSAIDs). The subsequent section outlines the most prevalent complications associated with peptic ulcers (Proctor & Deans, 2014):

- *Hemorrhage:* All ulcers cause little bleeding due to the erosion of tiny blood vessels, which is possible to be detected by examining the stool for hidden blood. The likelihood of bleeding from an ulcer sore increases if it develops on a major artery. While a major hemorrhage results in bloody vomit and/or stools, a mild blood loss leaks plasma slowly and leaves the person feeling lightheaded plus uncomfortable. The majority of bleeding patients are older than 60 and on NSAIDs. In addition, 15% of ulcer patients experience bleeding (Laine, 2016).
- *Perforation:* Perforation occurs when an ulceration penetrates deeply into the stomach or duodenal wall, allowing acid of the stomach and other contents to escape into the normally aseptic peritoneal cavity. This results in inflammation and infection of the peritoneum, leading to sudden, intense, and severe pain in patients. In some cases, patients could potentially progress to septic shock, a critical life-threatening medical state that necessitates abrupt surgical intervention. Perforation is observed in about seven % of patients, with a death risk of approximately 19% in the global residents (Dadfar & Edna, 2020).
- *Penetration*: When an ulcer sore breaks through the duodenum's or stomach muscular wall and travels into a neighboring organ, such as the liver or pancreas, this is known as penetration. The afflicted organ gives the sufferer intense, piercing agony (Haubbich, et al., 1953).
- *Obstruction:* Pyloric stenosis is the result of a fibrous scar developing at or close to the pylorus. It happens once an ulcer sore, expanding from inflammatory material, or ulcer scar prevents food from leaving the stomach and going through to the duodenum. Such a problem manifests as bloating, an appetite loss, weight loss, and even vomiting. Surgery may be required to treat obstructions in some cases (Zittel, et al., 2000).

2.5 Prevalence of Peptic Ulcer

Peptic ulcer disease represents a global public health challenge due to its significant impact on health, mortality, and economic burden, compounded by the widespread prevalence of Helicobacter pylori infection. It ranks among the most prevalent gastrointestinal disorders worldwide, affecting approximately 10-15% of the global population (Lauret & Rodrigo, 2015).

Notably, 19 out of every 20 peptic ulcers are of the duodenal type. Tragically, an estimated 15,000 lives are lost annually due to peptic ulcers. Incidence estimates for peptic ulcer bleeding and perforation range from 19.4 to 57 and 3.8 to 14 per 100,000 individuals, respectively. On average, there is a 13.9% recurrence rate of bleeding within seven days and a 12.2% long-term recurrence rate of perforation (Lau, et al., 2011). Surgical cases of peptic ulcer illness in Africa's Sub-Saharan region reveal that 86% involve duodenal ulcers, with the remaining 14% being gastric ulcers (Arito, 2018).

A study conducted at Al-Basrah teaching hospital and Al-Shafaa general hospital in Basrah City, Iraq in order to assess the prevalence of peptic ulcer revealed that out of 476 patients included in the study, 51.7% were endoscopically diagnosed to have peptic ulcer. Revealing high prevalence of peptic ulcer among Iraqi (Jaccob, et al., 2021).

2.6 Causes of Peptic Ulcer

The two most frequent causes of peptic ulcers worldwide are NSAIDs, such as aspirin, as well as H. pylori infection. Globally, H. pylori septicity is still the principal source of peptic ulcers, but in developed nations, the infection is less common. NSAIDs, which make up over 25% of the causes of peptic ulcers, are the main culprits after H. pylori. In these nations, the principal cause of H. pylori infections will likely be surpassed within a generation or two by the use of NSAIDs for age-related illnesses (Majumdar & Bebb, 2019).

Helicobacter pylori is a significant bacterial agent responsible for chronic infections that lead to chronic gastritis, peptic ulcers, and gastric cancer in the human gastrointestinal system. Its contribution to ulcer formation is linked to the stimulation of excessive gastrin secretion. Gastrin indirectly enhances acid secretion by influencing histamine and relies on the gastric antral mucosa and the intraluminal pH for regulation (Liu et al., 2022). The presence of gastrin in the antrum is associated with an anti-inflammatory response, disrupting the body's defense mechanisms, which can predispose individuals to ulcer formation (Jaiswal, et al., 2019). Furthermore, H. pylori, a Gram-negative bacillus bacterium, produces multiple virulence factors, facilitated by the large quantity of urease it contains. This enzyme catalyzes the conversion of urea into ammonia and carbon dioxide, allowing the bacterium to survive in acidic conditions. It generates a substance that buffers the acidity surrounding the bacteria (Ansari & Yamaoka,

2017). While H. pylori colonization does not invade the mucosa directly, it causes damage by secreting proteins that trigger cellular and humoral immune responses, leading to macrophage invasion of the mucosa and the development of chronic superficial gastritis, ultimately resulting in ulcer formation (Baj, et al., 2020). H. pylori spreads from person to person through the oral or fecal-oral pathways. In poorer nations, the chance of contracting H. pylori infection is decreasing. Compared to thirty years ago, the rate of infection in the United States has decreased by more than fifty percent (valle, 2022). Additionally, it's important to note that smoking alone does not directly cause peptic ulcer formation. However, when combined with H. pylori infection, it can impede the healing process by affecting the balance between factors that contribute to and protect against ulcers. Cigarette smoke hinders the healing process by reducing blood flow to the edges of the ulcer, leading to a decrease in the activity of constitutive nitric oxide synthase which plays a crucial role in widening blood vessels and enhancing blood supply to the gastric mucosa. The reduction in the enzyme activity contributes to the development of ulcers (Ma, et al., 1998). NSAIDs are among the most widely prescribed drugs both internationally and domestically. Around 30 billion over-the-counter tablets are sold each year. Prostaglandins are essential for preserving the integrity and healing of the gastroduodenal mucosa. As a result, disruption of prostaglandin synthesis may worsen mucosal defense and repair, which may facilitate mucosal injury through a systemic pathway. In addition, Damage to the mucosa is a consequence of the local application of NSAIDs, resulting in an increase in the permeability of the epithelial surface. Both aspirin and numerous NSAIDs are characterized as weak acids and, within the acidic environment of the stomach, exist in a nonionized, lipophilic state. In this state, NSAIDs can traverse the lipid membranes of epithelial cells, causing cell damage once they become trapped in an ionized form inside the cells. The application of topical NSAIDs can also modify the surface mucous layer, allowing for the back diffusion of hydrogen ions (H+) and pepsin, resulting in further injury to epithelial cells (valle, 2022).

2.7 Risk Factors for Peptic Ulcers

Several vulnerability reasons are connected with the expansion of peptic ulcers. Yet, the main factors are stated below (Rosenstock, et al., 2003) (Lau, et al., 2011):

- Helicobacter pylori Infection: This bacterium is a major cause as well as a risk factor of peptic ulcers. It weakens the protective mucous layer of the stomach and duodenum, making them more susceptible to damage from stomach acid.
- Regular use of NSAIDs: like aspirin and ibuprofen, can irritate the stomach lining, potentially leading to ulcers. Long-term, multiple NSAIDs use or high-dose use increases the risk.
- Excessive Alcohol Consumption: can irritate and erode the stomach lining, increasing the risk of ulcers.
- Smoking: An increased risk of peptic ulcers is linked to smoking, since it may interfere with the stomach's ability to defend against the damaging effects of stomach acid.
- Age: Peptic ulcers are more common in older adults.
- Family History: A family history of peptic ulcers may increase the risk.
- Prior Ulcer: people having an ulcer in the past, are at higher risk of developing another.
- Stress: While stress alone doesn't cause ulcers, it can exacerbate symptoms in individuals already affected.
- Zollinger-Ellison Syndrome: This rare condition involves tumors in the pancreas or duodenum that cause excess production of stomach acid, leading to ulcers.
- Serious Illness or Injury: Severe illness, injury, or major surgery can increase the risk of stress ulcers.
- Concomitant use of glucocorticoids: a potent anti-inflammatory medications can weaken the body's natural defenses against peptic ulcer development and may stimulate the secretion of gastric acid, further contributing to the risk of ulcer formation.

It's important to note that not everyone with these risk factors will develop peptic ulcers, and many people with ulcers have no identifiable risk factors. Lifestyle modifications, such as quitting smoking and moderating alcohol consumption, can help reduce the risk of peptic ulcers (Ohlsson, 2017).

2.8 The Effects of Free Radicals and Oxidation Load on Gastric Ulceration

Free radicals formed from oxygen, damage the structural strength of biological tissues. Lipid peroxidation is the apparatus of impairment, causing subsequent tissue damage by rupturing cell membranes and releasing internal elements such lysosomal enzymes. By producing DNA damage, a total change in cell metabolism, and the breakdown of the components of the epithelial basement membrane, radicals also contribute to mucosal injury. Moreover, lipid peroxidation contributes to the development of gastric lesions by impairing ion transport, membrane integrity, as well as fluidity of the covering on the surface of epithelial cells (Demir, et al., 2003).

On the other hand, the human body has evolved various endogenous antioxidant systems to counteract the generation of reactive oxygen species (ROS). Antioxidants function as radical scavengers, inhibiting processes like lipid peroxidation and other free radical-mediated reactions. This protective mechanism guards against diseases associated with radical reactions (Repetto & Llesuy, 2002) (Dokmeci, et al., 2005). These antioxidants can be categorized into enzymatic and nonenzymatic groups. Enzymatic antioxidants include superoxide dismutase (SOD), the primary antioxidative enzyme, along with catalase and glutathione peroxidase, which operate as a coordinated system to shield the body from the harmful effects of free radicals. These enzymes rely on trace metal co-factors, such as selenium for glutathione peroxidase, copper, zinc, or manganese for SOD, and iron for catalase, to achieve maximum efficiency (Nasuti, et al., 2006). Furthermore, Vitamins E and A that are soluble in fat, as well as water-soluble vitamins C and glutathione (GSH), are examples of non-enzymatic antioxidants. The intracellular synthesis of glutathione from cysteine, glycine, and glutamate allows it to scavenge reactive oxygen species directly or through the action of glutathione peroxidase (Demir, et al., 2003).

Different systems, such as SOD, peroxidase, catalases, and tissue thiol groups, are present in oxygen-handling cells and can shield thecells from the harmful effects of free radicals, of which

$O2^{-}$• is one of the most destructive (Repetto & Llesuy, 2002). Toxic substances are kept out of the stomach and duodenum by a number of mucosal defense systems. Yet, damage to the stomach mucosa may be caused by reactive oxygen species (ROS) released during the breakdown of arachidonic acid, clotting cells, immune cells, and smooth muscle fibers (Nasuti, et al., 2006).

2.8.1 The Impact of Ethanol on the Mucosal Membrane of the Stomach

The acute administration of ethanol leads to an increase in the production of reactive oxygen species (ROS), specifically superoxide (O2•-) and hydroxyl radicals (OH-), as well as oxidative degradation of lipids in the mucosa of the GIT, resulting in mucosal destruction. Chronic ethanol dosing in animal models, on the other hand, leads to additional cell proliferation. The damage caused by ethanol is believed to occur through a direct impact on gastric epithelial cells, which results in lipid peroxidation or the generation of oxidative shock within the cell. Prostaglandin administration or intracellular antioxidants like glutathione can prevent this damage, suggesting that superoxide free activists play a role in the development of ethanol-induced mucosal damage of the stomach while also highlighting the protective function of these innate chemicals against ethanol-induced harm to gastric cells (Repetto & Llesuy, 2002).

In animal models, giving extreme ethanol concentrations such as 90% v/v to 100% has often been employed to assess gastric lesions effectively. Further investigations involving rats endangered with short ethanol administration directly to the stomach and tobacco smoke have exemplified a Symbiotic unfavorable consequence on the gastric mucosa due to reduced blood arrival to the mucosa, aggravated irritation, and amplified production of unstable molecules (Siegmund et al., 2003) (Shams & Eissa, 2022).

The activation of phagocytes in the gastric mucosa also causes oxidative stress and the physiological effects of acute ethanol intoxication, resulting in the creation of O2•-, H_2O_2, $NO^{\cdot}$, and HOCl. This activation is pursued by the release of arachidonate and the fabrication of peroxides via enzymes, such as lipoxygenase and cyclooxygenase production. Peroxides give rise to alcoxyl (RO.) and peroxyl radicals (ROO.), which can harm other proteins and lipids.

Additionally, damage to the mitochondria leads to an increased transfer of electron, thereby, generates O2•-. Elevated intracellular calcium levels, along with the induction of several activities like nuclease and calcium-dependent nitric oxide synthase activities, further contribute to the risk of oxidative stress and damage to the gastric mucosal membrane (Siegmund et al., 2003).

Figure 2.8.1 provides a visual depiction that illustrates the impacts of both acute and chronic ethanol exposure on the stomach in a comprehensive manner (Siegmund et al., 2003).

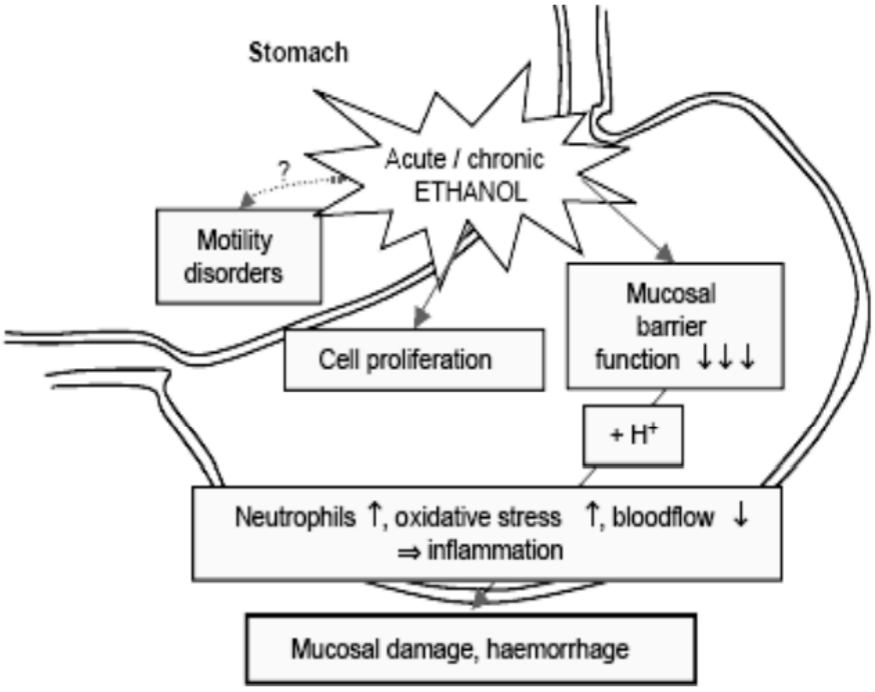


Figure 2.8.1: *Illustration of the effects of ethanol, both short-term and long-term, in the abdomen (Siegmund et al., 2003).*

2.9 Approaches for Treating Peptic Ulcers

According to Jain and his fellows (2007), treatment for peptic ulcers frequently aims to either increase the stomach mucosa's defense mechanism or reduce aggressive elements. These treatment approaches range from the practice of straightforward over-the-counter antacids to the more sophisticated and potent usage of proton pump inhibitors (PPIs). Additionally, according to

Chang et al. (1996), adding an antibiotic to the treatment plan is essential for treating peptic ulcers linked to Helicobacter pylori (H. pylori). But the negative consequences that these drugs are known to have are starting to raise questions. For example, long-term use of proton pump inhibitors that has irreversible action suppresses acid production, which upsets the stomach mucosa's natural physiology. Severe acid suppression at recommended dosages can occasionally result in achlorohydria and increase the risk of developing enteric illnesses such as dysentery, cholera, and typhoid (Jain, et al., 2007). Currently, there is interest worldwide in the quest for natural materials with therapeutic qualities, especially those derived from floras and Honey-producing bees that have fewer harmful anti-ulcerogenic attitudes and can be utilized as an alternative to or in addition to contemporary medications (Silva, et al., 2018).

2.9.1 Standard Peptic Ulcer Therapies

Before 1970, the primary approach to alleviate peptic ulcer pain involved the use of antacids and bismuth (Ippoliti & Peterson, 1979). However, in contemporary times, there is a wide array of potent drugs and drug combinations available that not only relieve pain but also enhance the stomach's protective mechanisms, eliminate H. pylori infections, and facilitate ulcer healing. Nevertheless, it's important to note that many of these medications come with the potential for side effects (Haastrup, et al., 2018). For example, antacids can lead to elevated blood pH, expulsion of gas, nausea, abdominal distension, constipation or diarrhea (Maton & Burton, 1999). On the other hand, anti-secretory drugs like pirenzepine may result in parasympathetic side effects, including dry mouth, blurred vision, and constipation (Feinberg, 1993).

Furthermore, ulcer treatment approaches and medications can be categorized into three main groups (Jain, et al., 2007):

1) Drugs such antacids, H2 receptor antagonists, and proton pump inhibitors (PPIs) that either counteract stomach acids or prevent them from being produced.
2) Prescriptions and natural remedies that enhance the protective mechanisms of the gut against the potential injury caused by gastric acid, including sucralfate, prostaglandin analogs, and bismuth.
3) Antibiotics that target the eradication of H. pylori bacteria.

Antacids are primarily designed to provide temporary relief from the noticeable symptoms of gastric distress by counteracting the effects of HCl secretion in the stomach. However, they are not the most effective treatment for peptic ulcers. Using antacids to self-medicate for pain relief when ulcers occur does not address the underlying issue, and, in many cases, the condition worsens over time (Helpern, 2004).

Contemporary medical approaches to treating peptic ulcers often focus on reducing gastric acid secretion using H2-receptor antagonists, proton pump inhibitors, and anti-muscarinic drugs. Additionally, acid-independent therapies, like sucralfate and bismuth, are utilized. In an acidic gastric environment, these medications create a glycoprotein-bismuth complex at the site of erosion and ulcerative lesions that acts as a protective barrier that hinders the backflow of H+, thereby expediting the healing process of erosions and wounds (Djanaev, et al., 2023). In addition, the recommended medication for people with hyperphosphatemia (uremia) is sucralfate (Begg, et al., 2023).

Yet, one significant challenge in treating gastroduodenal ulcers with H2-antagonists and proton pump inhibitors is the relatively high rate of ulcer recurrence within one year after discontinuing treatment, which can range from 40% to 80% (Helpern, 2004).

2.9.2 Omeprazole as a Drug of Choice

PPIs have steadily gained prominence in the treatment of acid-related conditions since the introduction of omeprazole in 1989 (Strand, et al., 2017). These drugs function by being activated in an acidic environment within parietal cells, allowing them to become ionized and establish covalent disulfide bonds with cysteine present in the H+-K+-ATPase. Once the PPI attaches to the proton pump, it effectively deactivates the pump, preventing the release of H+ ions. The structure of PPIs is a key factor contributing to their effectiveness (Ward & Kearns, 2013). On the other hand, H2 Receptor Antagonists (H2RAs) reduce the secretion of H + ions by parietal cells.

In addition, current clinical guidelines advocate for PPIs as the primary choice for gastroprotection medications, and this recommendation is reinforced by systematic reviews and

meta-analyses conducted in clinical contexts (Scally, et al., 2018). However, there has been no extensive endeavor to compare the efficacy of PPIs and H2RAs in addressing treatment-resistant or refractory peptic ulcers. It's important to note that H2 blockers have been withdrawn from the market due to their adverse side effects, including an elevated risk of gastric cancer (McGwin, 2020).

Additionally, as previously mentioned, The most effective drugs for reducing acid secretion are the PPIs, which include omeprazole, esomeprazole, pantoprazole, lansoprazole, and rabeprazole. Omeprazole is the first example of a novel kind of antiulcer medication that prevents the stomach's parietal cells' proton pump (N+, K+-ATPase) from working. Since omeprazole is a weak base, it is inert in a pH-neutral environment. However, because of disulfide linkages, it interacts irreversibly with the membrane's N+, K+-ATPase in the acidic environment of the parietal cell channels, changing into the active metabolite sulfenamide. This explains why omeprazole has a very specific effect on parietal cells, which have the environment needed for sulfenamide production. This conversion occurs rapidly, usually within 2-4 minutes. Sulfenamide is a cation that lacks absorption characteristics and omeprazole is therefore regarded as a pro-drug (Djanaev, et al., 2023).

Although not 100% effective, PPIs have been shown to be effective in preventing ulcers caused by various factors, particularly those induced by NSAIDs. Their effectiveness is attributed to a reduction in gastric juice acidity, which affects the damage caused by NSAIDs to the gastric mucosa. Drawing from the available data on the mechanism of harmful action, it can be inferred that PPI's capacity to avert stomach mucosal injury is linked to a reduction in the harsh characteristics of gastric juice, thereby influencing the injured tissues resulting from the use of NSAIDs (Kavitt, et al., 2019). Studies with high adherence to omeprazole treatment have demonstrated a significant reduction in the risk of gastric bleeding observed during long-term NSAID and antithrombotic drug treatment (Pasha, & Divya, 2022).

On the other hand, activated omeprazole's administration in experimental rats was found to suppress the release of prostacyclin secreted by the gastric fundus and at high doses eliminated the release of PGE2 (100), increasing sensitivity of the duodenal mucosa to ethanol's toxic effects. It's interesting to note that the duodenal mucosa of rats given omeprazole at a dose of 10 mg for every one Kg for a period of 29 calendar day were more susceptible to the harmful effects

of ethanol. The impact is definitely mediated by prostaglandins, as evidenced by the fact that indomethacin exacerbates it; nevertheless, omeprazole has no effect on PGE2 production (Armah, et al., 2021).

PPIs generally have low toxicity and do not induce withdrawal symptoms as observed with H2 histamine blockers. In addition to their anti-secretory effects (El-Din, et al., 2021), PPIs also neutralize the long-term adverse effects of anti-inflammatory steroids, anticoagulants, and antiaggregants (Li, et al., 2021). Due to their excellent efficacy and minimal adverse effects, antisecretory medications have improved the efficiency of prophylactic anti-secretory therapy from 3% to 40–60% during the past ten years (Narayanan, et al., 2018).

It's also important to note that PPIs are prescribed more than necessary in some statistical studies, especially among elderly patients due to their effectiveness, safety, and affordability (Begg, et al., 2023). However, long-term use of these drugs requires periodic monitoring of gastric mucosa condition due to the development of compensatory hypergastrinemia. There is evidence that PPIs can enhance the development of atrophic gastritis and adenocarcinoma, particularly in individuals with H. pylori-associated atrophic gastritis. The state of the mucous membrane was not affected by intragastric injection of 100 mg of omeprazole for every one kg of healthy animals in Mongolian gerbil tests. Nevertheless, H. pylori-associated atrophic gastritis has been demonstrated to promote the development of adenocarcinoma (Tadesse, et al., 2022). Moreover, long-term PPI usage has been coupled with an augmented hazard of osteoporosis. For instance, at 12 months, femur bone mineral density significantly decreased in individuals receiving esomeprazole treatment (Aleraij, et al., 2020). Rats treated with omeprazole at doses of 20 or 40 mg/kg for 4 weeks have shown a slowdown in bone resorption, possibly due to digestive tract-related calcium absorption disorders since reduced stomach acidity can hinder the absorption of many micronutrients from food, including calcium and magnesium, which can lead to nutritional deficiencies and increased susceptibility to various diseases (Djanaev, et al., 2023).

Furthermore, omeprazole is widely recognized for its high effectiveness in treating conditions such as duodenal ulcers, gastric ulcers, esophagitis with peptic ulcers, and Zollinger-Ellison syndrome. It is primarily prescribed for these specific medical purposes (Begg, et al., 2023).

However, PPIs can lead to various side effects, including nausea, headache, dizziness, diarrhea, constipation, abdominal discomfort, cough, abdominal and shoulder pain, skin rashes, occasional

decrease in sexual potency, and gynecomastia. In extremely rare cases, there may be an increase in transaminase activity. The long-term use of these inhibitors can potentially lead to the overgrowth of microorganisms in the previously relatively "sterile" stomach and intestinal mucosa, including the translocation of H. pylori. Prolonged usage may also affect the bone-marrow hematopoietic system (Fu, et al., 2022).

2.9.3 Herbal Remedies as a Substitute for Treating Peptic Ulcers

Traditional healing techniques have been employed to address all medical conditions for the majority of human history (Helpern, 2004). According to estimates from the World Health Organization, almost 80% of people worldwide who live in underdeveloped nations rely on traditional plant medicines for their basic medical requirements where a significant majority of these medications are plant isolates or their active ideals (Sampson, et al., 2000). Therefore, since ancient times, people have employed plants and herbs to treat a variety of conditions, including gastrointestinal problems such as peptic ulcers. In addition to western medicine, hospitals in China also practice traditional Chinese medicine. Noting that all medical professionals in Germany receive training in the use of herbs (Helpern, 2004).

Moreover, the past several decades have been marked by research on the physiological impacts of chemicals present in foods and herbal treatments, as well as a growing interest in the significance of compounds that originate from plants for safeguarding human health. Similarly, given the numerous side effects associated with modern medications, there is a growing interest in exploring indigenous remedies with fewer adverse effects as potential alternatives for treating peptic ulcers (Bafna, et al., 2004). Recent efforts have focused on identifying novel anti-ulcer drugs from natural sources. Examples of such drugs include carbenoxolone from Glycyrrhiza glabra, solon from sophoradin, and gefarnate from cabbage (Rodriguez, et al., 2006). Liquorice, derived from the tuber and underground stem of various Glycyrrhiza glabra collections, involved a long history of use in medicine for its anti-ulcer properties. Its primary active component, a triterpenoid saponin, plays a key role in its ability to protect against ulcers (Borrelli & Izzo, 2000). Another natural supplement, zinc-carnosine, is composed of zinc and L-carnosine. It works by enhancing protective layers of the gastric mucosa and leveraging the stomach's innate

capacity to combat ailment, fight infections, and support the healing process. L-carnosine, which is a dipeptide composed of L-histidine and β-alanine, possesses free radical scavenger properties that contribute to its shielding and healing outcomes (Helpern, 2004). The therapeutic traits of traditional medicinal herbs are often accredited to the existence of various composites such as flavonoids, phenolic acids, tannins, antioxidants, coumarins and certain essential minerals like copper (Cu), manganese (Mn), and zinc (Zn). These secondary metabolites found in plants have demonstrated the ability to neutralize harmful free radicals, making them promising candidates for therapeutic purposes. As a result of their free radical scavenging properties, plant-derived antioxidants are considered valuable in safeguarding the gastric mucosa from oxidative damage and potentially expediting the healing of gastric ulcers (Repetto & Llesuy, 2002).

It is known that intragastrically administered gastroprotective substances derived from plants play a role in protecting the mucosal lining against a range of irritants and ulcer-inducing factors. These substances may exhibit inflammation suppression features by inhibiting the neutrophil and cytokine replies within the gastrointestinal tract and support tissue restoration by promoting the formulation of various growth factors. Additionally, they demonstrate antioxidant properties by hunting ROS and possess anti-nucleolytic, anti-carcinogenic and necrosis prevention behaviors (Liu et al., 2002) (Bankova, 2005). One such example is Rhubarb that has a long history of medicinal use, particularly in TCM. It has been employed for its potential therapeutic properties, including digestive benefits. This historical use provides a foundation for exploring its modern applications as a potential anti-ulcerogenic agent (Kolodziejczyk & Liudvytska, 2021).

2.10 History of *Rheum Rhabarbarum*

Rheum L., commonly known as rhubarb, is a genus of perennial herbs in the Polygonaceae family that includes roughly sixty species. Rhubarbs are well recognized as edible and/or medicinal plants around the world, especially in Asia (Pourjabali, et al., 2017). *Rheum rhabarbarum*, has a rich history and taxonomy. Here's an overview (McNeill, et al., 2003):

Taxonomy:

Kingdom: Plantae

Order: Caryophyllales

Family: Polygonaceae

Genus: Rheum

Species: R. rhabarbarum

History:

The leaves are poisonous because of a high oxalic acid concentration, and the petioles or stalks are frequently utilized in cooking (Slaughter, et al. 2012).

Ancient Use: The history of rhubarb dates back thousands of years. Its medicinal use can be traced to ancient China, where it was used in traditional Chinese medicine and Tibetan medicine for its laxative properties where Rhubarb root was one of the first herbal remedies used in Chinese medicine (Hao & Jiang, 2015).

Introduction to Europe: Rhubarb was introduced to Europe via the Silk Road, where it became highly sought after. Its roots were considered valuable for their medicinal properties. The ancient Greeks and Romans used it as a medicinal plant (Ciriacono, 2019).

Culinary Use: In medieval Europe, rhubarb's use shifted from primarily medicinal to culinary. It was used in recipes for pies, tarts, and jams, often sweetened with sugar. Rhubarb became a popular ingredient in desserts (Smith, 2007).

Cultivation in England: Rhubarb cultivation in England began in the 17th century. The plant's adaptability to the English climate led to its popularity. By the 19th century, rhubarb was a common kitchen garden plant (Lee, et al., 2017).

North American Cultivation: Rhubarb was brought to North America by European settlers. It thrived in the northern regions of the continent, and its culinary use continued to expand (Turner & Aderkas, 2012).

Modern Use: Today, rhubarb is cultivated worldwide and is well-known for its use in culinary dishes, particularly desserts like pies and crisps. However, it is essential to note that only the rhubarb stalks are edible, as the leaves contain toxic compounds (Slaughter, et al. 2012)..

In summary, Rheum rhabarbarum, or rhubarb, has a long history of use, transitioning from ancient medicinal applications to a beloved ingredient in modern cuisine. Its taxonomy places it within the Polygonaceae family, and it is closely related to other species within the Rheum genus.

2.11 Biological and pharmacological activities of *R. rhabarbarum*

As mentioned before, Rhubarb, or Rheum rhabarbarum, has been used medicinally for a long time, mostly for its root. Rheum rhabarbarum has been used in traditional Chinese medicine for thousands of years to cure a variety of ailments, including edema, gastritis, enteritis, and other conditions. Here are some of the medicinal uses of Rheum rhabarbarum:

2.11.1 Menopausal complaints

Thus far, isoflavones derived from the soy bean have been mostly linked to the phrases "phytoestrogens" and "phytoestrogenic activity." However, over the past 20 years, a greater knowledge of the Hormone-mimetic effects of distinct types of phytochemicals and the discovery of different suppliers of phytoestrogens have been achieved. Additionally, the field of research on the hormonal impacts of plant metabolites has expanded dramatically since it was discovered that isoflavones do not exhibit just estrogenic activities (Dietz, et al. 2016) (Sirotkin & Harrath, 2014). Data indicating a positive contribution of phytoestrogens in the prevention of several illnesses affecting women, including bone fragility and cancer of the breast, further heighten scientific and therapeutic curiosity in medicines with estrogenic properties of herbal origin (Obi, et al. 2009). Human liver cancer cells transiently transfected with ERα, ERβ, and ERE-reporter plasmid demonstrated a binding affinity to certain stilbene derivatives, such as piceatannol 3′-O-β-D-xylopyranoside, cis-rhaponticin, and rhapontigenin 3′-O-β-D-glucopyranoside, isolated from the roots of *R. rhabarbarum* (Park, et al. 2018). However, historically, perimenopausal symptoms have been treated with rhubarb root extract and it was also used in traditional remedy for menstrual abnormalities and discomfort (Hasper, et al., 2009).

2.11.2 Antioxidant properties

One of the most extensively studied properties of naturally occurring extracts obtained from plants is their antioxidant activity because oxidative stress plays a major role in the pathophysiology of many diseases. Non-biological experiments were used to evaluate the garden rhubarb's antioxidant assays in an initial step. Natural antioxidants found in R. rhabarbarum are able to fight off oxidative damage at various cellular levels of antioxidant defense. Its bioactive components, such as nitric oxide, superoxide anion, and hydroxyl radical, can function as scavengers of reactive oxygen species as well as regulators of gene expression and cell signaling pathways (Kalpana, et al., 2012). The stilbenes potential to boost the actions of the transcription factor known as the nuclear factor erythroid-derived 2-like 2 and the Nrf2-mediated tracks is one of the molecular mechanisms underlying these effects. Six stilbenes that were extracted from the rhizome of *R. rhabarbarum*, such as rhaponticin, resveratrol, desoxyrhaponticin, rhapontigenin, desoxyrhapontigenin, and isorhaponticin significantly decreased the amount of ROS that were produced intracellularly in macrophages (Choi, et al., 2014). In addition, seven plant compounds protected hepatocytes from oxidative stress in vitro caused by iron ions and arachidonic acid. The molecular processes underlying this advantageous activity have been linked to the AMP-activated protein kinase pathway, that represent an essential regulator of cellular metabolism and energy balance (Dong, et al., 2015).

2.11.3 Anti-inflammatory properties

The suppression of pro-inflammatory pathways dependent on nuclear factor kappa B is one of the molecular mechanisms of action of compounds produced from rhubarb. A *R. rhabarbarum* aqueous extract inhibited the activation of nuclear factor kappa B p65 in human umbilical vein endothelial cells by tumor necrosis factor α. It also lowered the production of adhesion molecules and monocyte chemo attractant protein-1 (Moon, et al., 2006). Macrophages' generation of nitric oxide was found to be inhibited by rhizome-derived stilbenes, their derivatives, and a naphthalene glucoside (Matsuda, et al., 2000). Aloe-emodin was also shown in other research on

macrophages to be capable of suppressing the pro-inflammatory state (Hu, et al. 2014). Accessible findings suggest that stilbenes extracted from this plant's tubers may reduce an inflammatory response brought on by allergies (Matsuda, et al. 2004). Kim et al. (2000) described rapontigenin as a suppressor mast cells liberation of histamine, hyaluronidase activity, and passive dermic anaphylactic response. Desoxyrhapontigenin demonstrated anti-inflammatory effects in and outside the living system. Additionally, it might function as an instinctive controller or stimulator of the hemeoxygenase enzyme, which is a crucial antioxidant, cytoprotective, and anti-inflammatory enzyme (Joo Choi, et al. 2014). Desoxyrhapontigenin is thought to exert its anti-inflammatory and anti-osteoporosis properties via blocking the NF-κB receptor activator ligand (RANKL) (Tran, et al. 2018).

Adding to the above described findings, anti-inflammatory qualities of R. rhabarbarum extracts have also been observed in investigations involving animals and humans. An animal prototype for intestinal damage caused by lipopolysaccharide (LPS) during sepsis showed that rhein at a dose of 100 mg of every kg of the body weight suppressed the toll-like receptor 4 pathway (Zhang, et al., 2015). An ointment made of *R. rhabarbarum* rhizome extract with a dose of 1 mg for every one g was used twice a day to treat 120 appendectomy patients in a randomized clinical trial. This treatment dramatically decreased inflammation and accelerated the healing of sutures. According to Li et al., (2016), complementary studies in this work on human endothelial cells of the umbilical vein revealed the pro-angiogenic activity of the rhubarb extract under investigation.

2.11.4 Cardioprotective Properties

The majority of the time, the biological activity of many naturally occurring chemicals is clearly beyond the antioxidant and/or anti-inflammatory actions that are principally responsible for the cardioprotective benefits of plant-derived substances. The etiology and pathophysiology of cardiovascular problems are complicated and diverse, encompassing a wide variety of variables. As such, research on contemporary preventative and therapeutic measures is necessary. Natural compounds provide cardioprotective qualities that include antithrombotic effect and anti-clotting activity, improvement of the lipid profile in the blood and relaxation of blood vessels, in addition to their antioxidant and anti-inflammatory activities.

When R. rhabarbarum was given to rats fed a high-cholesterol diet, the serum lipid level declined in a dose-dependent manner while the level of high-density lipoprotein (HDL) cholesterol increased significantly. Furthermore, administration of either rhaponticin or rhapontigenin dramatically decreased pathological alterations in the fatty liver that was degenerating (Jo, et al., 2014). This plant has been shown to possess endogenous suppressors of the soluble epoxide hydrolase enzyme, which serves as a crucial molecular objective for the treatment of cardiovascular disease. Using a recombinant human enzyme, preliminary investigations showed that the methanol isolate, n-hexane, butanol and chloroform portions from *R. rhabarbarum* inhibited it to varying degrees with methanol extract achieving the best inhibition (Jo, et al., 2016). Both studies conducted inside and outside the human body revealed that compounds produced using *R. rhabarbarum* had anti-obesity and hypolipidemic properties. According to Lee et al. (2010), a hot water extract from this plant inhibits protein tyrosine phosphatase 1B, a crucial regulator of the insulin signaling pathway. Research using mice given a high-fat diet showed that rhubarb extracts and its anthraquinone constituents, such as physcion and chrysophanol, had the ability to regulate metabolism. The extract considerably decreased the rise in body weight in rats when given daily for eight weeks. This included modulating the animals' lipid metabolism and stimulating the manufacture of adiponectin (Lee, et al. 2012). Additionally, there is some minimal evidence available regarding the anti-thrombotic activity of *R. rhabarbarum* stilbenes. In vitro, blood platelets forced to aggregate by collagen or arachidonic acid were inhibited by desoxyrhapontigenin and rhapontigenin, whereas piceatannol showed no antiplatelet effect (Ko, et al. 1999). In addition to the actions listed above, *R. rhabarbarum* may also have anti-diabetic and vasorelaxant properties. The top portions of *R. rhabarbarum* may encompass mild repressors of α-glucosidase, according to an in vitro screening of extracts from 24 different plants for this enzyme's inhibitory activity. The enzyme was inhibited via the methanol preparations made from the stripped stalks and peel (Kongstad, et al. 2015). An aqueous extract of *R. rhabarbarum* rhizomes was found to exhibit in vitro vasodilatory characteristics in rat aorta preparations (Moon et al. 2006). Furthermore, this extract's vasorelaxant and anti-inflammatory properties were verified in vivo in rats fed an atherogenic diet. Significant anti-atherogenic benefits of the rhubarb extract treatment included a decrease in low-density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol in blood plasma (Moon, et al., 2008).

2.11.5 Anticancer Activity

The chemopreventive and antitumor properties of plant extracts, including the stilbene and anthraquinone components, have been documented in previous studies. However, the anticancer potential of extracts derived from rhubarb has mainly been assessed in vitro. In one study, a methanol extract from *R. rhabarbarum* triggered apoptotic cell death in human adenocarcinoma gastric cells through the activation of the intrinsic apoptotic pathway, which is mitochondria-dependent (Hong, et al. 2015). These cells' exposure to either chrysophanol 1-O-β-D-glucopyranoside or aloe-emodin caused the anti-apoptotic protein Bcl-2 to be downregulated and poly (ADP-ribose) polymerase to be cleaved. Additionally, according to Trinh et al. (2019), chrysophanol 1-O-β-D-glucopyranoside marginally increased the expression of pro-apoptotic proteins including Bax and Bid.

Lee, et al. (2018) demonstrated that Chrysophanol 1-O-β-D-glucopyranoside functioned via the mitochondria-dependent apoptotic pathway, but aloe-emodin and rhapontigenin triggered death through the mitochondria-independent caspase-8 pathway in the human breast cell line MCF-7. Desoxyrhapontigenin also triggered cancer cell apoptosis in the same experimental model. Its anticancer effect was characterized by endoplasmic reticulum (ER) stress marker expression rising and ER expansion (Venkatesan, et al. 2016).

Furthermore, a hexane extract derived from *R. rhabarbarum* considerably inhibited the proliferation and survival of cancer cells and triggered apoptosis in the oral cancer cell lines HN22 and SCC15 (Choi et al. 2011).

2.11.6 Antimicrobial and Antiviral Activities

Li et al., (2016) have demonstrated that the hydrophobic hydroxyanthraquinone, emodin, may alter the structure of membrane proteins, increasing membrane permeability and potentially destroying bacterial cell membrane integrity. Hydroxyanthraquinones are the primary active

ingredients in rhubarb roots, and they have a variety of biological and pharmacological characteristics, including antibacterial qualities.

Very little is known about rhubarbs' antiviral characteristics (Nurbaulina et al. 2009). The primary difficulties are connected to the lack of in vivo investigations, and although there have been some positive results, estimating the pharmacological importance of rhubarbs' antimicrobial effect is challenging. Additionally, a lot of the time beneficial effects were only seen at high, physically impossible quantities. It is known, however, that a variety of plant formulations are used externally, such as to burns, wounds, or ulcers. Higher dosages of bioactive compounds might also be therapeutically helpful in certain circumstances, provided the required in vivo testing are carried out. Preparations including *R. rhabarbarum* root materials have long been consumed to treat tooth ailments, it has been confirmed by recent research that this plant has a protective function in maintaining oral health by inhibiting the growth of dental plaque and the formation of glycolytic acids by two Streptococcus species, the mutans and the sobrinus. (Kim, et al. 2011). Rhein, one of the roots of *R. rhabarbarum's* anthraquinone components, is thought to be potentially helpful in preserving dental health because it exhibits the power to resist Gram-negative periodontopathogen like Porphyromonas gingivali, a (Chinsembu, 2016). Furthermore, it has been shown that rhein and metronidazole antibiotic may work in concert (Azelmat, et al. 2015).

2.11.7 Other Biological Activities

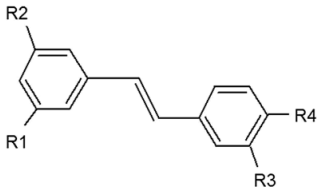
Rhein, a significant flavonoid derived from *Rheum rhabarbarum*, contributes to the regulation of tight junctions and the preservation of intestinal barrier function through various pathways. Rhein's ability to restore intestinal barrier function is attributed to its NF-κB/MLCK/p-MLC pathway blockage (Zhuang, et al., 2019). In an in vitro model involving LPS-stimulated IEC-6 cells, rhein was observed to normalize the expression and distribution of ZO-1, mitigate a rise in phenol efflux that is red, with an attenuated reduction in TEER. Additionally, it reduced phosphorylation of MLC, expression of MLCK, and NF activation of κB sutype. Furthermore, a decreased in the secretion levels of IL that is of 1β subtype and IL number 6 is achieved by rhein molecule, accompied by a downregulation of the TLR4 and NLRP3 molecules expression, as

well as caspase1 cleavage inhibition, and suppressed NF-κB expression. Mechanistically, rhein's protective effects on the epithelial layer of the intestines were achieved by blocking the NF-κB/MLCK/p-MLC path, while it restrained the expression of IL-1β and IL-6 in response to LPS by the NLRP3 inflammasome and TLR4/NF-κB pathway modification.

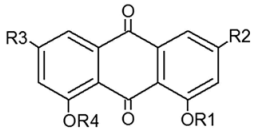
Moreover, rhein has been recorded to shield the gut barrier against oxidative and inflammatory damage induced by LPS by suppressing p38 MAPK and JNK, thereby inhibiting their activity, and activation of the Nrf2 pathway. The administration of rhein prior to intraperitoneal LPS injection resulted in a noteworthy elevation in intestinal ZO-1 and occludin levels in a rat model. Rhein also successfully reduced intestinal and inflammatory cells and NO levels in the blood, which in turn reduced LPS-induced intestinal soreness and oxidative stress. It simultaneously increased intestinal catalase and glutathione enzymatic peroxidase capabilities, elevated HO^{-1} appearance, and lowered intestinal malondialdehyde measures (Zhong, et al., 2019).

2.12 *R. rhabarbarum* Phytochemical Profile

Research on the phytochemical makeup of various rhubarb species dates back to the early 1900s and 42 components were discovered. These studies revealed the presence of a range of anthraquinones, such as emodin, aloe-emodin, and rhein, and stilbenes, as well as organic and acids that are not organic, such as oxalic, citric, tartaric, malic, and ascorbic acid (Pucher, et al. 1938) (Tutin & Clewer, 1911). Different garden rhubarb cultivars may change slightly in their polyphenolic makeup and the amount of various chemicals (Rumpunen & Henriksen, 1999). However, some significant groups of compounds are typically found in the roots and petioles of all cultivars, including torachysone, anthraquinones (emodin, chrysophanol, and their glucosides), and trans-stilbenes (resveratrol, piceatannol, rhapontigenin, and their O-glucosides) (Kolodziejczyk & Liudvytska, 2021). According to Komatsu et al., (2006), the main flavonols found in petioles include quercetin, epicatechin, epigallocatechin gallate or gallocatechin gallate, myricetin-O-rhamnoside, quercetin-O-rutinoside (rutin), quercetin-O-glucuronide, quercetin-Oglucoside, and quercetin. Depending on the cultivar, the anthocyanins that can be found in the petioles are cyanidin-3-O-glucoside and cyanidin-3-Orutinoside (Takeoka et al., 2013). Figure 2.12 represents the primary chemicals found (Kolodziejczyk & Liudvytska, 2021).



Stilbene compounds	R1	R2	R3	R4
Desoxyrhapontigenin	OH	OH	H	OCH_3
Isorhapontigenin	OH	OH	OCH_3	OH
Piceatannol	OH	OH	OH	OH
Pterostilbene	OCH_3	OCH_3	H	OH
Rhapontigenin	OH	OH	OH	OCH_3
Resveratrol	OH	OH	H	OH



Antraquinone compounds	R1	R2	R3	R4
Aloe-emodin	H	CH_2OH	H	H
Emodin	H	CH_3	OH	H
Chrysophanol	H	CH_3	H	H
Physcion	H	CH_3	OCH_3	H
Rhein	H	COOH	H	H

Figure 2.12: *The primary antraquinone and stilbene constituents found in Rheum rhabarbarum (Kolodziejczyk & Liudvytska, 2021).*

2.13 Plant Extraction

The extraction methods employed play a crucial role in the analysis of phytochemicals. These methods encompass both traditional and advanced techniques. Extracting bioactive compounds from Rheum rhabarbarum (rhubarb) typically involves various methods, such as decoction, maceration, and percolation. However, the choice of extraction method depends on the specific compounds researchers aim to extract and the intended application (Azwanida, 2015).

Decoction: This traditional method involves boiling dried or fresh rhubarb roots and stems in water. Decoction is suitable for extracting water-soluble compounds like anthraquinones and other active constituents. It is often used for making herbal teas or tinctures (Tandon & Rane, 2008).

Maceration: In maceration, chopped or ground rhubarb plant parts are soaked in a solvent such as water, alcohol, or oil for an extended period. This allows for the gradual extraction of both water-soluble and alcohol-soluble compounds. Maceration is commonly used in the preparation of herbal medicines (Singh, 2008).

Percolation: In order to prepare tinctures and fluid extracts, percolation is a more efficient method involving the passage of a solvent through a powdered rhubarb sample. An appropriate quantity of the specified mixture is used to moisten the solid components, which are then placed in the upper part of the percolator and allowed to sit for approximately four hours in a tightly covered container. Additional menstruum is introduced to create a thin layer over the mass, and this mixture is then left to macerate for one day in a sealed container. The percolator's outlet is opened to allow the liquid to gently flow out. Menstruum is added as necessary until the percolate reaches about three-quarters of the required final volume. The liquid obtained from pressing the marc is combined with the percolate, and enough menstruum is added to reach the desired volume. The combined liquid is then clarified either through filtration or by allowing it to stand before draining. This method is suitable for large-scale extractions and is often used in industrial settings to efficiently extract specific compounds (Malik & Singh, 2022).

Soxhlet Extraction: Soxhlet extraction is employed to extract compounds using a continuous cycle of solvent evaporation and condensation. It is efficient for extracting lipophilic compounds from plant materials (Azwanida, 2015).

Steam Distillation: This method is suitable for extracting essential oils from rhubarb. Steam is passed through the plant material, vaporizing the essential oils, which are then condensed into a liquid (Chemat & Boutekedjiret, 2015).

Supercritical Fluid Extraction (SFE): uses supercritical fluids, often carbon dioxide, to extract compounds. This method is efficient and is especially useful for extracting compounds with a low boiling point. It is commonly used in the food and pharmaceutical industries (Sasipriya, et al., 2013). The extraction process offers several distinct advantages:

- It enables the extraction of constituents at low temperatures, effectively preventing any damage caused by heat or certain organic solvents.
- There are no residual traces of solvents left behind.
- This extraction method is environmentally friendly.

The rapid expansion of supercritical fluid extraction (SFE) applications has been most notable in the realm of development. SFE is widely utilized in extracting pesticides, environmental samples, various foods and fragrances, essential oils, polymers, and natural products. However, the primary obstacle to its widespread commercial adoption is the substantial initial capital investment required for the extraction process (Singh, 2008).

Microwave-Assisted Extraction (MAE): MAE harnesses microwave energy to heat the solvent and expedite the extraction process. It can be a quicker method compared to traditional techniques. This approach utilizes microwave energy to simplify the extraction of active components from plant materials and their transfer into the solvent. Microwaves generate both magnetic and electric fields that are perpendicular to each other. Heat is produced by the electric field through ionic conduction and dipolar rotation. This heating is fast and depends on the dielectric constant of the solvent. In contrast to conventional methods, microwave-assisted extraction uniformly heats the entire sample at once. The heat generated during extraction disrupts fragile hydrogen bonds due to the molecular dipole rotation, and ion migration aids in the penetration into the sample or structure (Mandal, et al., 2007).

2.14 Animal Models Used for Studying Inflammation in the Intestines

Numerous mammalian models have been employed to investigate both acute and chronic inflammation of the intestines. Since mice's intestinal development resembles that of humans' and because they share many genes and immunological responses, they are regarded as useful animal models. Rat models have the benefit of being larger than mice, which makes it possible to get larger samples for examination. Because non-human primates' intestines are so close to humans' in terms of both genetic makeup and physiology, they offer the most accurate and comparable data to humans (Jiminez, et al., 2015). Various models of experimental ulcer have been created in order to look into the origins of the illness and assess the effectiveness of novel treatment approaches. Four main categories can be used to group these experimental models: chemical, bacterial, immunological, and genetic (Elson, et al., 1995) (Yan et al., 2009).

Genetic models: The two primary genetic models being utilized more and more to study the impact of host-related variables on the development of disease are gene deletion and transgenic models like specific components of the innate and adaptive immune system, such as IL-10 deficiency (Hoffmann et al., 2009).

Immunological models: These models are employed to examine the function of particular immune cell subpopulations in the pathophysiology of inflammation. In immunological models, immunodeficient mice are given a particular subtype of immune cells to study their functions and consequences in the development or management of disease (Ding, et al., 2005).

Bacterial models: These models examine the possible positive or negative effects of various bacterial strains on the development or treatment of intestinal inflammation by exposing germ-free, particular pathogen-free, or immunodeficient mice to a particular bacteria, pathogen or commensal (Nell, et al., 2010).

Chemical models: The ethanol-induced gastric ulcer model involves administering ethanol to induce gastric ulcers in a rat model. This model is relevant for studying the gastroprotective effect of rhubarb because ethanol is known to cause acute damage to the gastric mucosa and mimic the effects of certain factors that lead to gastric ulcers in humans. The concentration, mode of administration, and existence of microorganisms in the lumen all affect how well a chemical agent cause's damage (Jiminez et al., 2015).

2.15 Staining Techniques

In the fields of histology and pathology, staining techniques are essential because they allow researchers and medical practitioners to see and evaluate tissue samples under a microscope. By highlighting particular elements of the biological specimens with different dyes or reagents, these techniques offer important insights on the composition, organization, and pathological changes of the specimens. There are numerous staining techniques employed with hematoxylin and eosin (H & E) possibly being the most used staining method (Black & Black, 2018).

2.15.1 Hematoxylin and Eosin (H & E) Stain

The basic dye hematoxylin gives the nucleus of cells and other acidic structures a blue or purple hue. On the other hand, the acidic dye Eosin leaves pink or ruby stains on the extracellular matrix and cytoplasm. This highly adaptable stain combination serves as the foundation for routine tissue evaluation, enabling pathologists to differentiate between various tissue types, spot anomalies, and reach a diagnosis (Cardiff, et al., 2014). Hematoxylin and Eosin techniques have been proven to be useful for studying most histopathological processes (Titford & Bowman, 2012). In the same way, the technique can be modified and is inexpensive and easy to implement. Hematoxylin and eosin, on the other hand, are ineffective since they cannot capture all of a substance's characteristics and require the application of specific stains (Musumeci, 2014).

2.15.2 The Periodic Acid Schiff (PAS) Stain

In histology, the Periodic Acid Schiff (PAS) stain is another essential method. It specifically targets polysaccharides, emphasizing in magenta the glycogen and other carbohydrate-rich components. For the detection of fungal pathogens, disorders involving glycogen storage, and the assessment of renal biopsies, PAS staining is especially useful. It relies on the periodic acid's oxidation of diol functional groups in carbohydrates, followed by the reaction with Schiff's reagent, resulting in a magenta or purplish color stain in the presence of these substances. PAS staining can be applied to a wide range of tissue types, making it versatile for routine histopathological examination and research studies. In addition to its diagnostic utility, PAS staining is crucial in various research applications, including understanding tissue composition and the distribution of carbohydrates in biological samples (Shedge, et al., 2020). However, it is not without limitations, such as its lack of specificity and potential for false positives, necessitating careful consideration of its results in the context of a broader histological evaluation. In addition, PAS staining involves several steps, including the use of periodic acid and Schiff's reagent, which can be technically challenging and require careful handling of chemicals (Pérez, et al., 2021).

2.15.3 Immunohistochemistry (IHC)

In histology and pathology, immunohistochemistry (IHC) is a potent technique that is used to visualize the presence, distribution, and localization of particular proteins or antigens inside tissues or cells. The utilization of antibodies labeled with a visible marker, like an enzyme or a fluorescent dye, is essential to this technique. These antibodies enable pathologists and researchers to recognize and examine the expression of particular molecules in biological samples by binding to their target proteins or antigens and producing a detectable signal. IHC is helpful in the diagnosis of cancer because it can show if cancer-specific markers are present or absent, which helps with tumor classification and patient outcome prediction (Duraiyan, et al., 2012).

2.15.3.1 Heat shock protein 70 (HSP 70) staining

A particular immunohistochemical method called heat shock protein 70 (HSP 70) staining is used in pathology and histology to identify and visualize the presence of heat shock protein 70 in biological tissues or cells. A family of proteins known as heat shock proteins is essential to cellular stress responses, particularly in situations where cells are subjected to high temperatures or other stresses. Particularly HSP 70 is well-known for its chaperone and protective properties while under stress (Tavassol, et al., 2011). One significant advantage of HSP 70 staining is its utility in assessing cellular responses to stressors, such as heat, toxins, or other environmental challenges. The expression and localization patterns of HSP 70 provide valuable information about cellular stress levels and the activation of protective mechanisms. Additionally, HSP 70 staining is frequently employed in cancer research to understand the role of these proteins in tumorigenesis and response to therapeutic interventions (Hanemoto, et al., 2019).

However, it's important to note some limitations associated with HSP 70 staining. One notable disadvantage is the potential for non-specific binding of antibodies, leading to false-positive results. Optimization of staining conditions and the use of validated antibodies are critical to obtaining reliable and accurate data. Interpretation of staining results also requires expertise, as various factors, including tissue type and experimental conditions, can influence the staining

patterns. Despite these challenges, HSP 70 staining remains a valuable tool in elucidating cellular stress responses and their implications in health and disease (Gehrmann, et al., 2014).

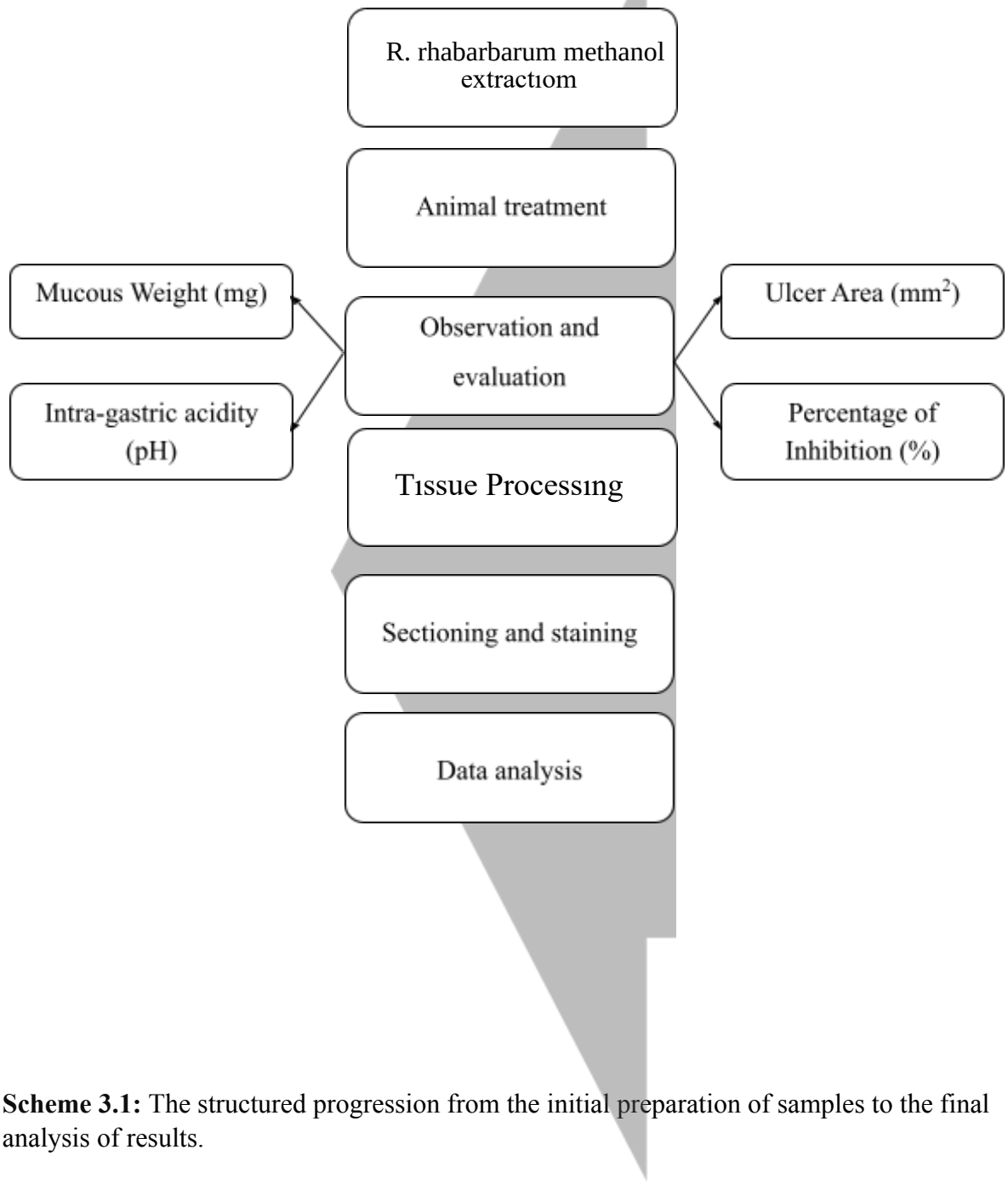
2.15.3.2 Bax staining

Bax staining is an immunohistochemical method for identifying and visualizing the presence of the Bax protein in biological tissues or cells that is utilized in pathology and histology. The pro-apoptotic protein Bax (Bcl-2-associated X protein) is essential for the control of apoptosis, or programmed cell death. The role of Bax in several biological processes and pathological states is better understood by researchers and pathologists thanks to this staining technique (Young, et al., 2003). However, Bax staining has inherent limitations, much like any other scientific procedure. A significant drawback is the possibility of non-specific antibody binding, which could result in false-positive readings. Obtaining ideal staining conditions and guaranteeing the specificity of the used antibodies are crucial obstacles in the process of Bax staining. Because staining patterns and intensity can change depending on a variety of factors, including tissue type and experimental settings, interpreting staining results also takes knowledge. Notwithstanding these difficulties, Bax staining continues to be an important technique in molecular and cellular research, furthering our knowledge of apoptosis and its consequences in a range of physiological and pathological processes (Bedner, et al., 1999).

Chapter III

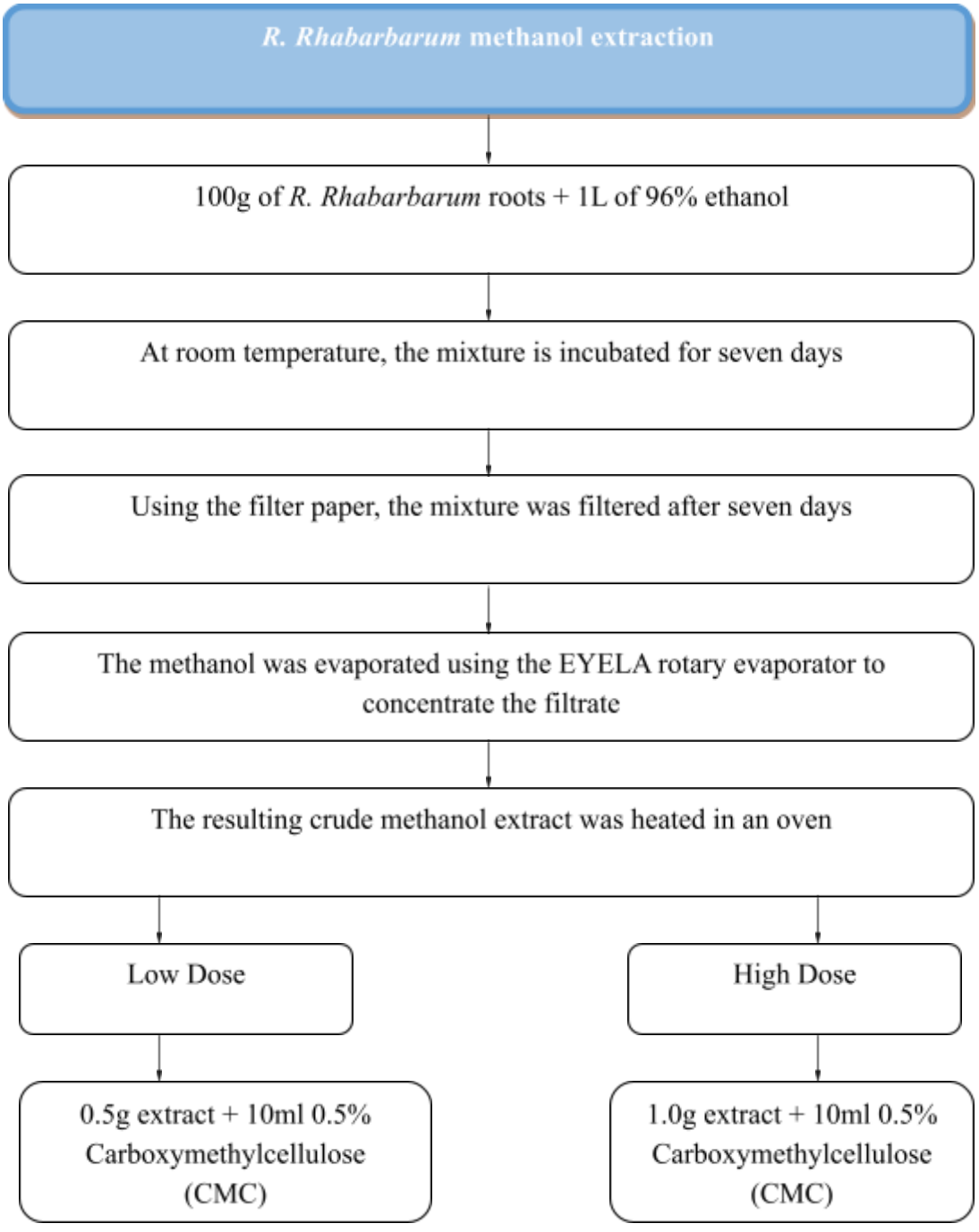
Material and Methods .3

The sequence of procedures 3.1



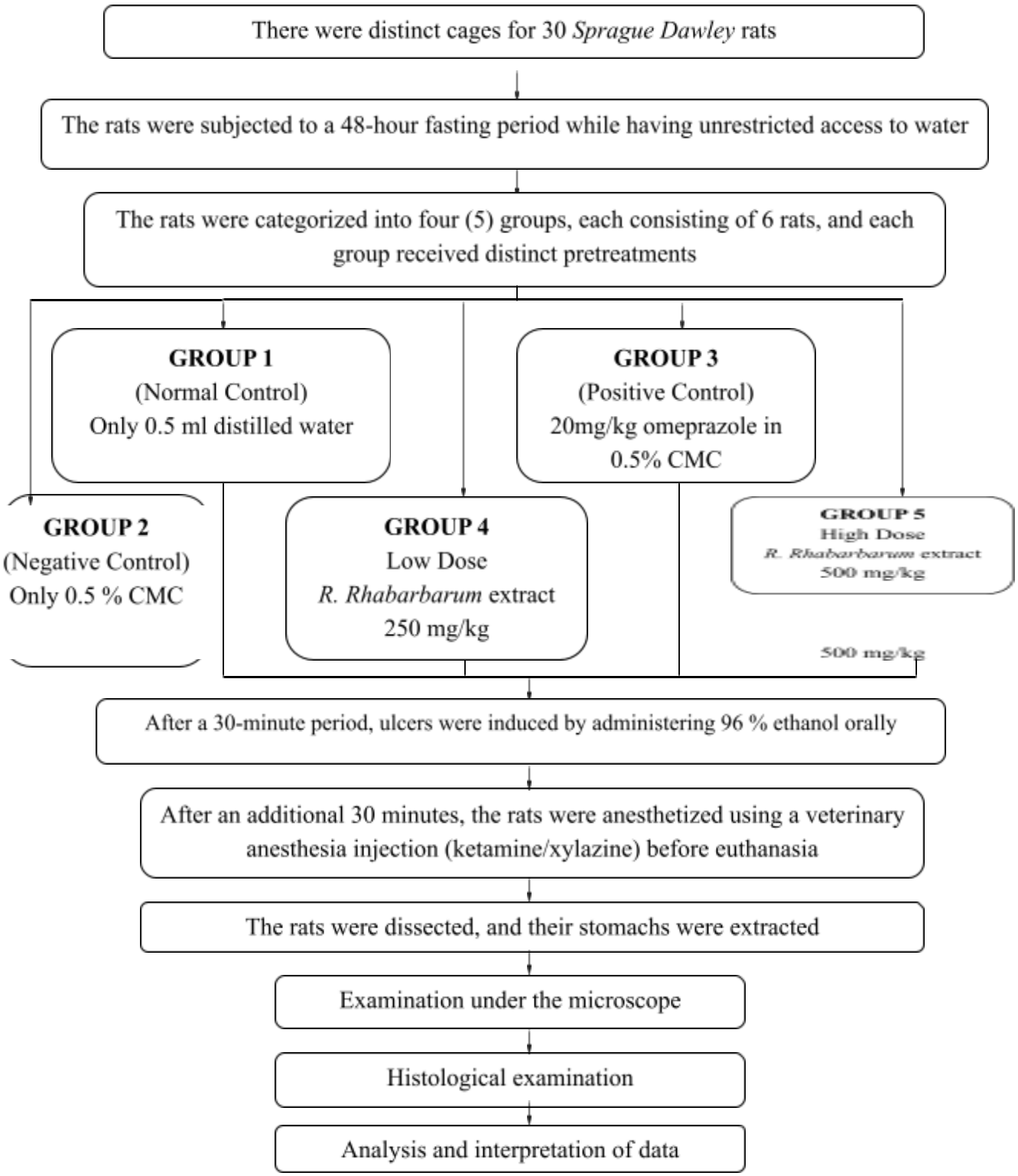
Scheme 3.1: The structured progression from the initial preparation of samples to the final analysis of results.

3.1.1 *R. Rhabarbarum* Methanol Extraction



Scheme 3.1.1: Preparation of *R. Rhabarbarum* root methanol extract.

3.1.2 Animal treatment preparation



Scheme 3.1.2: Illustration of the animal's treatment process.

Experimental Materials 3.2

3.2.1 Plant materials

R. Rhabarbarum roots were purchased from Erbil's local markets and transported to Cihan University's Biology Division in Erbil.The plant was identified by the roots were then reduced in size by grinding and crushing them with an electrical grinder. Rheum Ribes polygonaceae family is one of the most important medicinal plants spread wildly in Kurdistan Iraq, and its roots are used traditionally in the treatment.

Figure 3.2.1: *Rhubarb root.*

3.2.2 Experimental Animals

Thirty Sprague Dawley rats, males and females, with weights ranging from 180 to 200 grams were used in the experiments. These rats were procured from the Animal House, Faculty of Medicine, University of Cihan. Each rat was housed individually in a wide-mesh wire cage to prevent cannibalism and coprophagy (consumption of feces). The rats were kept in a controlled environment with a 12-hour light/dark cycle, maintaining a constant temperature of 25°C, along with standard conditions of lighting, humidity, and temperature. A 48-hour fast with unlimited access to water was imposed on the rats before to the experiment in order to minimize any potential cross-reaction between the stomach content and the medications that had already been administered. With six rats per group, the rats were divided into different experimental groups according to different pretreatments. The Iraqi Ministry of Health's (MOH) Guidelines for Handling Laboratory Animals were followed in all protocols and rat handling.

Figure 3.2.2: *The Sprague dawley rats.*

3.2.3 Carboxymethylcellulose (CMC)

Several solutions serve as suitable negative controls in this experiment, including phosphate-buffered saline (PBS), distilled water, carboxymethylcellulose (CMC), and 10% Tween 20. A robust negative control is crucial for any experiment, as it helps ensure that we are not assessing unrelated treatment effects, thereby minimizing the likelihood of obtaining false-positive results. In this study, CMC demonstrates superior ability in solubilizing and diluting *R. Rhabarbarum* extracts. Additionally, it proves effective in transporting the extract during the administration process.

3.2.4 Omeprazole

Prior research has indicated that proton-pump inhibitors like omeprazole and lansoprazole possess the capability to inhibit gastric secretion (Mullin, et al., 2009). In this study, omeprazole was selected as the positive control over lansoprazole due to its demonstrated superior effectiveness in inhibiting gastric acid secretion, as supported by Katz et al., (2001). The omeprazole used in the experiment was sourced from the University of Cihan pharmacy.

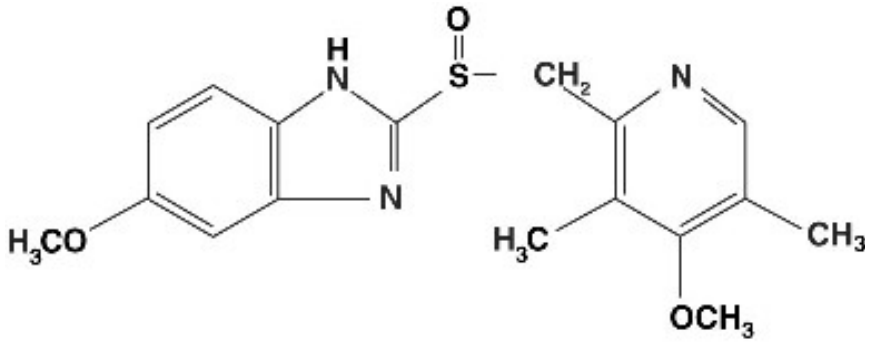


Figure 3.2.4: *The molecular structure of omeprazole.*

Experimental Studies 3.3

3.3.1 Preparation of *R. Rhabarbarum* methanol extracts

One hundred grams (100g) of dried and ground *R. Rhabarbarum* roots were immersed in 1L of absolute methanol within a Schott bottle. The mixture underwent a 7-day incubation period at room temperature. After this duration, the combination of the grounded *R. Rhabarbarum* roots and methanol was filtered using filter paper. The resulting filtrate underwent evaporation through the EYELA Rotary Evaporator to remove the methanol. The crude extracts obtained from this process were then transferred into a Universal bottle. This bottle was subsequently incubated in an oven with the cap loosened to allow for the evaporation of any remaining methanol in the extract, thereby reducing the methanol content. The extract was later diluted with 0.5% carboxymethylcellulose (CMC) to create two different doses: a low dose where 0.5g of the extract was mixed with 10ml of 0.5% CMC, and a high dose where 1.0g of the extract was diluted with 10ml of 0.5% CMC.

3.3.2 Preparation of Control Groups

Six naive rats were housed in a cage. They were given distilled water.

3.3.2.1 Negative Control

Six naïve rats were housed in a cage. They were given (CMC) was prepared by mixing 0.5g of CMC powder with 100ml of distilled water.

3.3.2.2 Positive Control

A 20mg omeprazole tablet was mixed with 10ml of 0.5% carboxymethylcellulose (CMC) to create a 20mg/kg omeprazole dose, which served as the positive control. 20mg Omeprazol was given to six rats.

3.3.3 Animal Treatment

30 Sprague Dawley rats were utilized in this experiment, and they were split into 5 groups of 6 rats each at random. To prevent fecophagy and anthropophagy, each rat was housed in a separate cage. Prior to treatment, the rats must fast for a whole day while having unlimited access to water. Two hours before to the treatment, the water was removed. Oral pretreatment then started, with each group receiving a distinct pretreatment as shown in Table 3.3.3 below.

Table 3.3.3: The pre-treatment administered to each group.

Group	No of rats	Pretreatment
1	6	0.5mL distilled water (normal control)
2	6	0.5% carboxymethylcellulose (CMC) (negative control)
3	6	20mg/kg omeprazole (positive control)
4	6	250mg/kg *R. Rhabarbarum* methanol extracts
5	6	500mg/kg *R. Rhabarbarum* methanol extracts

Every pretreatment, positive control, negative control, and 96 % ethanol received 5 milliliters per kilogram (i.e., 5 milliliters of the aforementioned treatments are injected for every kilogram of the rat's weight correspondingly). Rats were given 96 % absolute ethanol orally 30 minutes after the pretreatment in order to cause stomach injury (ulcers). The rats were given an intraperitoneal injection of veterinary anesthetic, a ketamine and xylazine combination, 30 minutes after the 96% absolute ethanol was administered. This anesthesia was administered to the rats in order to render them unconscious before their death. The rats' stomachs were then taken once they had

been dissected. In order to stop the stomach's mucus from spilling out, the pyloric and esophageal ends were tied off.

3.3.4 Gross Lesion Evaluation

3.3.4.1 Measuring the weight of gastric mucus

The gastric contents were squeezed from the stomachs and collected in separate universal containers corresponding to each rat. The containers with the gastric contents were then weighed, and the individual weights of the stomach mucous content were documented.

3.3.4.2 The measurement of intra-gastric acidity (pH)

The combined gastric mucous contents from the preceding step were pooled according to their respective groups. Subsequently, the gastric mucous contents underwent centrifugation, and the resulting supernatants were collected for pH measurement using a calibrated pH meter.

3.3.4.3 Ulcer area quantification

The stomachs, from which the contents were extracted, were dissected open along the greater curvature. The inner lining of the stomach was delicately rinsed to remove any remaining mucous. The ulceration area was assessed by examining the gross lesions under a dissecting microscope (with a magnification of x1.8), and the ulcer area was determined by counting the number of small squares (2mm x 2mm) that were completely covered by the ulcers (Sivaraman & Muralidharan, 2010).

$$\text{Ulcer Area } (mm^2) = \text{No of squares covered by ulcer} \times 4 \times 1.8$$

3.3.4.4 The percentage of Inhibition

The percentage of ulcer inhibition (%) is calculated by using the following formula (Sivaraman & Muralidharan, 2010):

$$Percentageofinhibition, I\% = \left(\frac{UA_{negativecontrol} - UA_{treatment}}{UA_{negativecontrol}}\right)x100$$

To demonstrate the anti-ulcerogenic effect of the treatments in the experiment, the percentage of ulcer inhibition is calculated. A higher percentage of inhibition indicates a more pronounced anti-ulcerogenic effect of the respective pretreatment.

3.3.4.5 Histological Examination

The stomachs, which were opened in the preceding step, underwent fixation in 10% buffered formalin for approximately 3 to 4 hours. Following this, the specimens were trimmed and placed in cassettes, which were subsequently immersed in 10% buffered formalin for an additional 2 days. The cassettes were then processed in an automatic tissue processor for approximately 5 hours. Subsequently, the tissues were embedded in paraffin and sliced into 5µm thick tissue sections using a microtome. These sections were then subjected to hematoxylin and eosin staining before being mounted with DPX. Finally, the slides were examined under a light microscope, and the pathohistological features of the slides were observed.

Toxicology study 3.4

Fifteen female and fifteen male rats were enrolled in the research study and subjected to a 48-hour fasting period with unrestricted access to water. Subsequently, the groups received

administrations of 2 g (low dose) or 5 g (high dose) of *R. Rhabarbarum*. Following an additional 30 minutes, the rats underwent anesthesia using a veterinary injection containing ketamine and xylazine before being euthanized. The animals were dissected, and blood samples were collected to assess kidney and liver biomarkers, as well as lipid profiles. Furthermore, kidney and liver .tissues were taken out for subsequent histological examination

Statistical Analysis 3.5

Ulcer indices data were reported as mean values ± standard error of the mean (SEM) and the ulcer inhibition was expressed as a percentage. Statistical distinctions among the treatments were determined using one-way ANOVA, followed by student's t-test, with statistical significance set at $p < 0.05$ and $p < 0.01$.

Chapter IV

Results .4

Assessment of the acute toxicity of the plant extract 4.1

The rats were orally administered an extract of the aerial parts of *R. rhabarbarum* at doses of 2.5 g/kg and 5 g/kg for a duration of 14 days. However, none of the rats exhibited mortality or demonstrated any signs of toxicity in the experiment. No indications of hepatotoxicity or nephrotoxicity were observed, as assessed through both histological examination and biochemical analysis (Fig. 4.1, Table 4.1, A, B, C and D). Moreover, there were no fluctuations in body weight, and no discernible physiological or behavioral abnormalities were observed at doses of 2.5 g/kg and 5 g/kg over the 14-day duration, in comparison to the control group treated with 10% Tween 20.

In term of kidney biomarkers, the sodium level was 145.15 mmol/L (± 0.52) in female rats that received the vehicle 10% Tween 20. Similarly female rats who received 2.5 g/kg of plant extract had a sodium level of 144.05 mmol/L (+ 0.42) and those who received 5g/kg of plant extract had a sodium level of 143.11 mmol/L (± 0.17). Therefore the level didn't change between the control and the experimental group number 5. The level of potassium was 4.36 mmol/L (± 0.33) in female rats that received the vehicle 10% Tween 20 and it remained constant in female rates who received 2.5 g/kg and 5g/kg of *Rheum rhabarbarum* extract, 4.31 mmol/L (± 0.04), 4.40 mmol/L (± 0.23) respectively. Chloride level was 103 (± 0.45) mmol/L in female rats that received the vehicle 10% Tween 20. Similarly it remained relatively unchanged in female rats who received 2.5 g/kg of plant extract 104 (± 0.61) mmol/L and those who received 5g/kg of plant extract, 105 (± 0.53) mmol/L. Urea level seem to be 5.25 mmol/L (± 1.15) in the vehicle 10% Tween 20 group. Yet the level slightly increased to 6.50 mmol/L (± 0.74) in female rats obtaining 2.5 g/kg of plant extract and to 4.05 mmol/L (± 0.33) in those who received 5g/kg of plant extract. Moreover, Creatinine level remained constant in control group and experimental group where it

was noted as 34.18 mmol/L (± 2.75) in 10% Tweet 20 group, 33. 63 mmol/L (+ 3.17) in 2.5 g/kg female rats group and 35.52 mmol/L (± 3.01) in 5g/kg female rats group (table 4.1 A).

The sodium level was 143.17 mmol/L (± 0.43) in male rats that received the vehicle 10% Tween 20. Similarly male rats who received 2.5 g/kg of plant extract had a sodium level of 142.13 mmol/L (± 0.23) and those who received 5g/kg of plant extract had a sodium level of 145.14 mmol/L (±0.19). Therefore the level varied in an unsignificant way between the control and the experimental group. The level of potassium was 4.33 mmol/L (± 0.35) in male rats that received the vehicle 10% Tween 20 and it remained relatively constant in male rates who received 2.5g/kg and 5g/kg of *Rheum rhabarbarum* extract, 4.31 mmol/L (± 0.08) and 4.42 mmol/L (±0.26) respectively. Chloride level was 103.26 (± 0.45) mmol/L in male rats that received the vehicle 10% Tween 20. Similarly it remained relatively unchanged in male rats who received 2.5 g/kg of plant extract 104.10 (± 0.61) mmol/L and those who received 5g/kg of plant extract, 105.00 (± 0.53) mmol/L. Urea level seem to be 5.85 mmol/L (±1.14) in the vehicle 10% Tween 20 group. Yet the level slightly increased to 6.52 mmol/L (± 0.72) in male rats obtaining 2.5 g/kg of plant extract and to 6.09 mmol/L (± 0.37) in those who received 5g/kg of plant extract which might indicate a slight effect on the liver. Moreover, Creatinine level remained constant in control group and experimental group where it was recorded as 35.13 mmol/L (±2.77) in 10% Tweet 20 group, 34.53 mmol/L (± 3.16) in 2.5 g/kg male rats group and 35.22 mmol/L (± 3.04) in 5g/kg male rats group (table 4.1 B).

In terms of liver biomarkers, female rats who received 10% Tween 20 had a total protein level of 78.05 g/L (± 2.50) similar to the female rats who were administered plant extract at 2.5 g/kg dose with a total protein of 77.35 g/L (± 0.33) and those who were administered plant extract at a dose of 5g/kg with a total protein level of 76.16 g/L (± 0.18). The albumin level was recorded as 12.31 g/L (+ 0.36) in female rats obtaining 10% Tween 20 compared to 11.36 g/L (± 0.24) in rats obtaining 2.5g/kg of plant extract and 13.42 g/L (± 0.25) in female rats obtaining 5g/kg of plant extract. ALP level was 98.04 IU/L (± 1.46), 95.24 IU/L (± 1.69) and 92.75 IU/L (± 1.58) in female rats receiving 10% Tween 20, plant extract at 2.5 g/kg dose and 5g/kg respectively. Furthermore, ALT level was 35.22 IU/L (±1.16) in female rats getting 10% Tween 20, and it remained slightly unchanged in respect to female rats who received 2.5 g/kg, 38.53 IU/L (± 1.72) and 5g/kg of plant extract 33.07 IU/L (± 1.36). Lastly, the AST level was recorded as 197.12

IU/L (±2.35) in female rats receiving10% Tween 20, 192.68 IU/L (±3.11) in female rats receiving 2.5 g/kg dose and 194.50 IU/L (± 3.31) in female rats receiving 5g/kg dose of plant extract (table 4.1 C).

Regarding liver biomarkers, male rats treated with 10% Tween 20 demonstrated a total protein level of 68.25 g/L (±2.53), comparable to those administered plant extract at a dose of 2.5g/kg, where the total protein was 67.15 g/L (± 0.37), and at a dose of 5 g/kg, with a total protein level of 66.36 g/L (± 0.17). The albumin level in rats receiving 10% Tween 20 was noted as 13.32 g/L (± 0.38), while male rats treated with 2.5 g/kg of plant extract had an albumin level of 12.38 g/L (± 0.22), and those receiving 5 g/kg exhibited 12.44 g/L (± 0.28). ALP levels were measured at 105.06 IU/L (± 1.49), 110.23 IU/L (± 1.42), and 108.15 IU/L (± 1.27) in male rats treated with 10% Tween 20, plant extract at a dose of 2.5 g/kg, and 5 g/kg, respectively. Moreover, ALT levels were 48.28 IU/L (±1.12) in male rats receiving 10% Tween 20, showing minimal increase compared to those receiving 2.5 g/kg (52.51 IU/L, ± 1.76) and 5 g/kg of plant extract (45.23 IU/L, ± 1.33). Finally, the AST level was recorded as 203.32 IU/L (±2.37) in male rats treated with 10% Tween 20, 207.62 IU/L (±3.15) in those receiving a 2.5 g/kg dose, and 211.52 IU/L (± 3.35) in those receiving a 5 g/kg dose of plant extract (Table 4.1 D).

Table 4.1 A: Effects of plant root extract on kidney biochemical parameters in female group of rats.

	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Normal range	136 - 145	3.5-5.0	96 - 106	6.4	35.3-70.7
Female Group Dosage					
Vehicle (10% Tween 20)	145.15 ± 0.52	4.36 ± 0.33	103 ± 0.45	5.25 ±1.15	34.18 ± 2.75
Plant extract 2.5 g/kg	144.05 ± 0.42	4.31 ± 0.04	104 ± 0.61	6.50 ± 0.74	33.63 ± 3.17

Plant extract 5 g/kg	143.11± 0.17	4.40 ± 0.23	105 ± 0.53	4.05 ± 0.33	35.52± 3.01

Values are expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at P<0.5.

Table 4.1 B: Effects of plant root extract on kidney biochemical parameters in male rats.

	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Normal range	136 - 145	3.5-5.0	96 - 106	6.4	35.3-70.7
Male Group Dosage					
Vehicle (Tween 20 10%)	143.17 ± 0.43	4.33 ± 0.35	103.26 ± 0.45	5.85 ±1.14	35.13 ± 2.77
Plant extract 2.5 g/kg	142.13 ± 0.23	4.31 ± 0.08	104.10 ± 0.61	6.52 ± 0.72	34. 53 ± 3.16
Plant extract 5 g/kg	145.14 ± 0.19	4.42 ± 0.26	105.00 ± 0.53	6.09 ± 0.37	35.22 ± 3.04

Values are expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at P<0.5.

Table 4.1 C: Effects of plant root extract on liver biochemical parameters in female rats.

	Total protein (g/L)	Albumin (g/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)

Normal range	60 - 83	34 - 54	30-130	10-40	50-150
Female Group					
Vehicle (10% Tween 20)	78.05 ± 2.50	12.31 ± 0.36	98.04 ± 1.46	35.22 ±1.16	197.12 ± 2.35
Plant extract 2.5 g/kg	77.35 ± 0.33	11.36 ± 0.24	95.24 ± 1.69	38.53 ± 1.72	192.68 ± 3.11
Plant extract 5 g/kg	76.16 ± 0.18	13.42 ± 0.25	92.75 ± 1.58	33.07 ± 1.36	194.50 ± 3.31

Values are expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at P<0.5.

Table 4.1 D: Effects of plant root extract on liver biochemical parameters in male rats.

	Total protein (g/L)	Albumin (g/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Normal range	60 - 83	34 - 54	30-130	10-40	50-150
Dose in male					
Vehicle (10% Tween 20)	68.25 ± 2.53	13.32 ± 0.38	105.06 ± 1.49	48.28 ±1.12	203.32 ± 2.37
Plant extract 2.0 g/kg	67.15 ± 0.37	12.38 ± 0.22	110.23 ± 1.42	52.51± 1.76	207.62 ± 3.15
Plant extract 5 g/kg	66.36 ± 0.17	12.44 ± 0.28	108.15 ± 1.27	45.23 ± 1.33	211.52 ± 3.35

Values are expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at P<0.5.

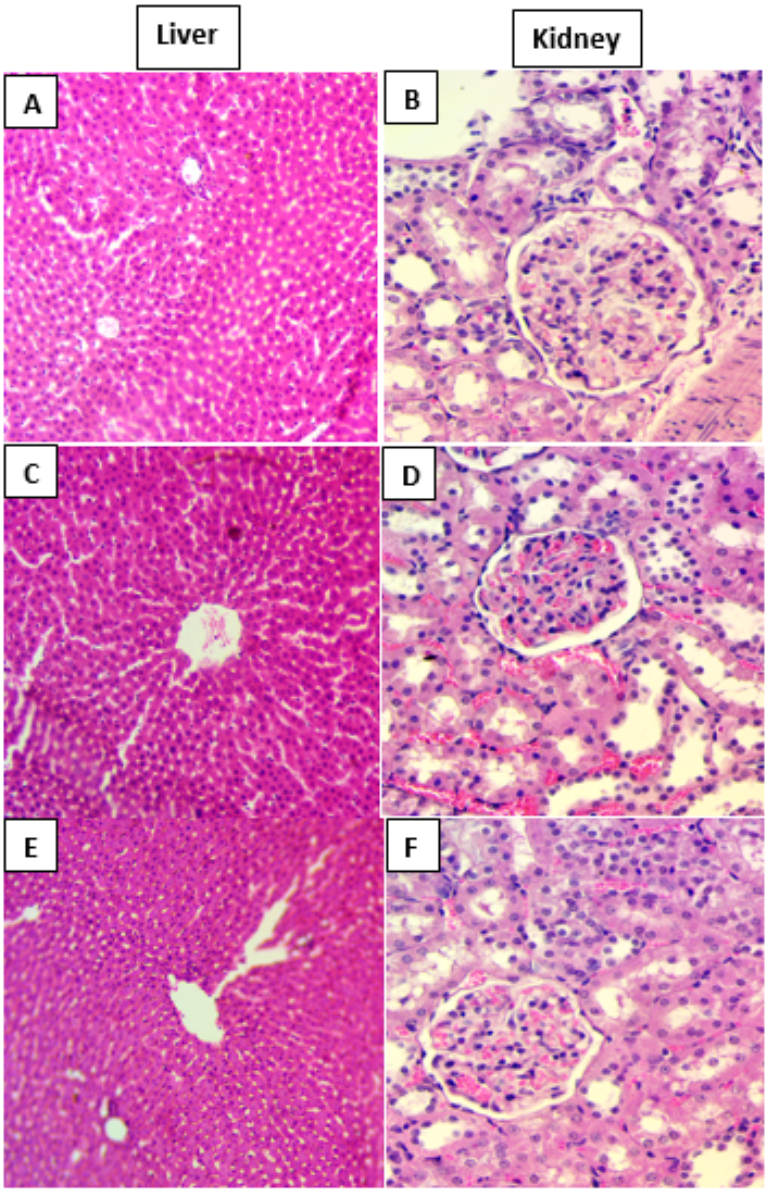
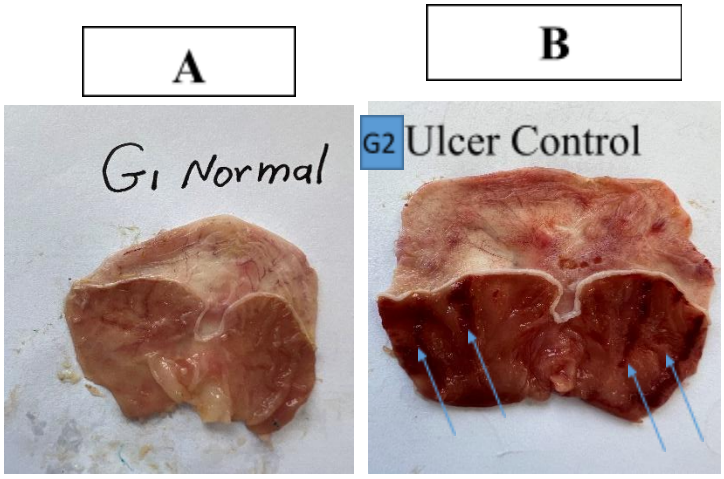


Figure 4.1: Histological slides depicting liver and kidney sections from the acute toxicity experiment. Sections from animals treated with 5 mL/kg of the vehicle (10% Tween 20) are labeled A and B. Sections from animals treated with 2500 mg/kg (2.5 g /kg) of the plant extract are labeled C and D. Sections from animals treated with 5000 mg/kg (5 g/kg) of the plant extract are labeled E and F. No notable alterations were observed in the liver and kidney structures among the treated groups and the control group. The staining used was hematoxylin and eosin, and the magnification was set to 20x.

Influence of *Rheum rhabarbarum* on Macroscopic Evaluation of the Stomach 4.2

The findings of this investigation indicate that the administration of absolute ethanol to induce peptic ulcers in rats leads to significant damage in the stomach, resulting in severe gastric lesions as depicted in Figure 4.2B (G2). Rats administered Rheum rhabarbarum displayed a noteworthy decrease in stomach ulceration compared to the ulcer control groups, as illustrated in Figure 4.2 B, D and E. The gross evaluation of the experimental rats' stomachs revealed a flattened epithelial surface and a reduction in mucosal damage in those treated with Rheum rhabarbarum, in contrast to the ulcer control groups. Moreover, The Omeprazole-treated group shows minor injuries to the gastric mucosal surface. 4.2 D & E. Experimental groups treated with Rheum rhabarbarum display a noticeable reduction in gastric mucosal ulceration. In addition, the ulcer area was 0.0 mm^2 in group one that represent the negative control in the study. However, it 911.83 mm^2 (± 33.51) in group 2 that represent the ulcer control. In group 3 the ulcer area decreased to 145.13 mm^2 (± 21.33) after being treated with omeprazole and it was decreased to 186.17 mm^2 (± 22.26) 163.51 mm^2 (±24.20) after being treated with 250 mg and 500 mg for .every one kilogram of body weight of *R. rhabarbarum* extract respectively as seen in table 4.2



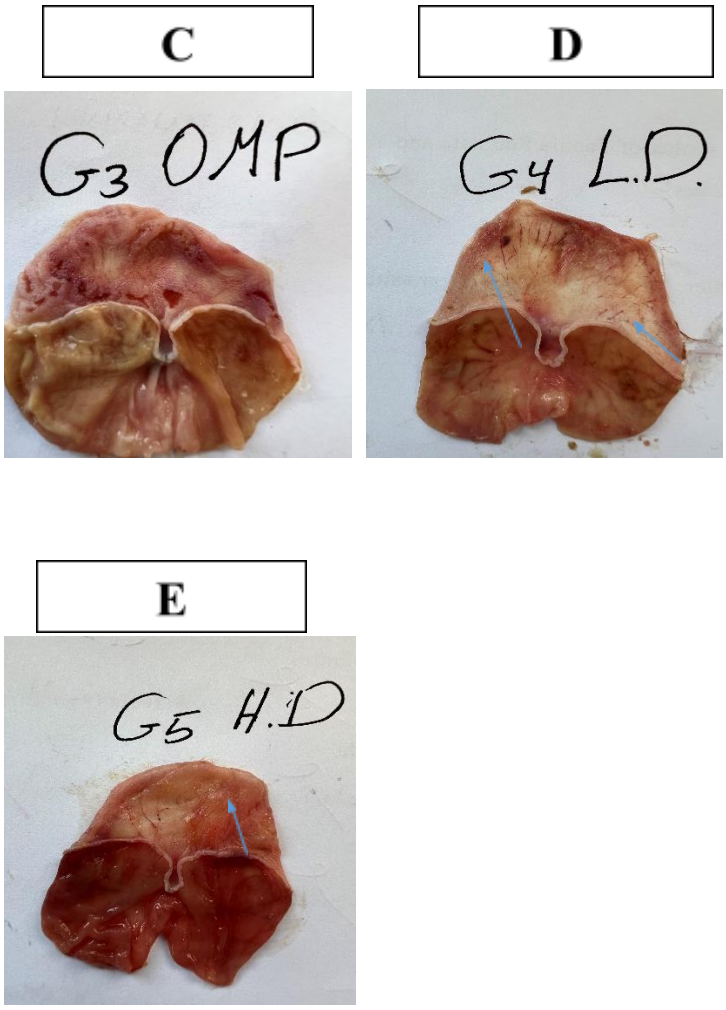


Figure 4.2: *Effect of Rheum rhabarbarum evident in macroscopic images of absolute alcohol-induced stomach injury in rats. A. Representative image of the normal control group displaying an intact stomach epithelium. B. The ulcer control group exhibits extensive hemorrhagic lesions on the stomach mucosa. C. The Omeprazole-treated group shows minor injuries to the gastric mucosal surface. D. & E. Experimental groups treated with Rheum rhabarbarum display a noticeable reduction in gastric mucosal ulceration.*

Table 4.2: Effect of R. rhabarbarum extract on induction of gross lesions in the absolute ethanol induced gastric ulcer model as well as the Intra-gastric acidity and Mucous Weight in the .different groups

Group	No. of animal	Pre-treatment (ml kg^{-1} 5)	Ulcer area (mm^2 Mean ± S.E.M)	tra-gastric acidity ((pH	Mucous Weight (gram)	Ulcer Inhibition (%)
1	6	0.5mL distilled water (Normal control)	0.0	5.32 ± 0.84	0.59± 0.15	0.00
2	6	0.5% CMC (Ulcer control)	911.83 ± 33.51[a]	0.61 ± 3.43	0.13 ± 0.36	0.00
3	6	20 mg/kg Omeprazole (positive control)	145.13 ± 21.33	6.13±0.87	0.18 ± 1.57	84%
4	6	*R. rhabarbarum* extract (250 mg/kg)	186.17 ± 22.26	0.74 ± 4.32	0.13 ± 1.11	79.5%
5	6	*R. rhabarbarum* extract (500mg/kg)	163.51 ± 24.20	5.03±1.02	0.11 ± 1.33	82%

All values are expressed as mean ± standard error mean (S.E.M); mean with different superscripts are significantly different ($p < 0.05$).

Effect of *R. rhabarbarum* on Gastric Mucus Content 4.3

The mucous weight in group one which represent the normal control was 0.59g (+ 0.15). However, the mucous content decreased to 0.36g (± 0.13) in group 2 rats that represent the ulcer control. In addition, the mucous content was 1.57g (± 0.18) in group 3 that received omeprazole 20mg. the mucous content in group 4 and 5 that received 250 mg/kg and 500 mg/kg of *R. rhabarbarum* extract was 1.11g (± 0.13) and 1.33g (± 0.11) respectively showed in table 4.2. The outcomes of this experiment indicate a significant improvement in the weight of mucus in rats treated with the floating part of *R. rhabarbarum* compared to the ulcer control group (G2). Ethanol treatment significantly decreased mucus secretion in ulcer control rats, resulting in mucus weight being expressively lower than that of the normal group (G1), group having .Omeprazole (G3), R. rhabarbarum 250 mg/kg (G4), and 500 mg/kg (G5) figure 4.2

Effect of R. rhabarbarum on pH of the stomach 4.4

The intra-gastric acidity reflected by pH measurement had a value of 5.32 (+ 0.84) in the negative control represented by normal gastric cell. However, the pH was significantly lower in group 2 that represent the ulcer control with a value of 3.43 (+ 0.61). In group 3 that received omeprazole the pH value was 6.13 (±0.87**)** whereas the pH was 4.32 (+ 0.74) in group 4 that received 250 mg/kg of *R. rhabarbarum* extract lower than the pH measured in group 5, 5.03 (+1.02) where rat received 500mg/kg of *R. rhabarbarum* extract showed in table 4.2. Therefore, the pH measurements of the gastric mucus in the rat experiments reveal that pretreatment with omeprazole (G3) and the aerial part extracts of *R. rhabarbarum* (250 mg/kg G4 and 500 mg/kg G5) significantly reduced ulceration and resulted in lower stomach acidity. Consequently, the pH of the gastric mucus in G3, G4, and G5 was significantly higher than that in the ulcer control group (G2). The pH levels of G5 and G3 appear to be nearly similar. According to Table 4.2, the .extract's effect seems to be dose-dependent

Effect of *R. rhabarbarum* on Ulcer Inhibition 4.5

The highest percentage of inhibition was achieved by omeprazole treatment where it resulted in 84% inhibition of ulcer, followed by high dose *R. rhabarbarum* extract (500mg/kg) which produced 82% inhibition in ulcer and lastly low dose *R. rhabarbarum* extract (250 mg/kg) .resulted in an inhibition percentage of 79.5%

Effect of *R. rhabarbarum* on Histology of the Stomach 4.6

4.6.1 H&E Stain

There were profound abrasion of the mucosal epithelium of the stomach and swelling, leukocyte ingression of the subcutaneous coat in the control group with ulceration, indicating significant damage to the stomach epithelium. Rats in the experimental groups provided with *Rheum rhabarbarum* exhibited a decrease in ulcerated area and a relative improvement in the stomach epithelium's defense, as well as a decrease in the submucosal layer's edema inflammatory cell .penetration as seen in figure 4.6.1

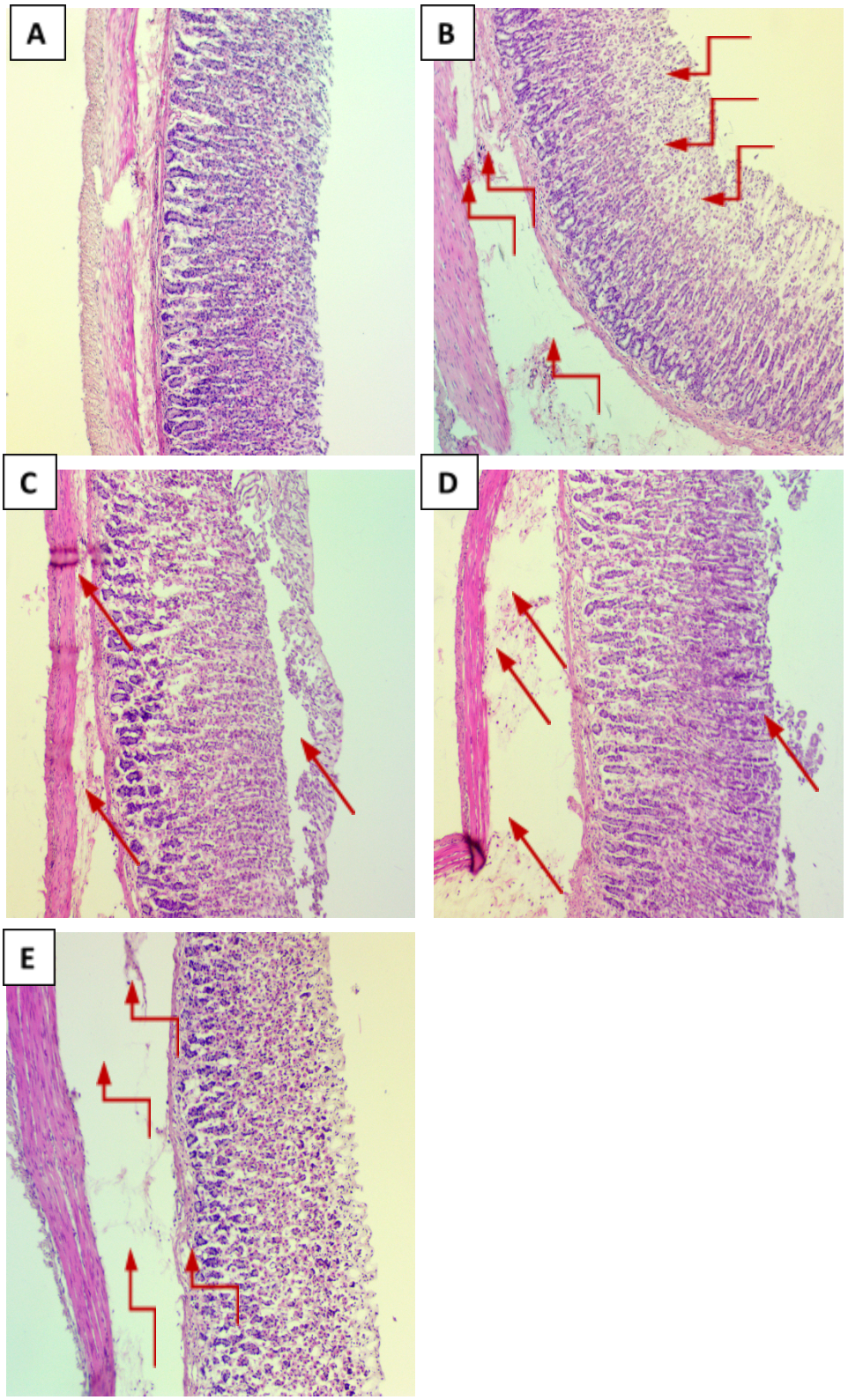
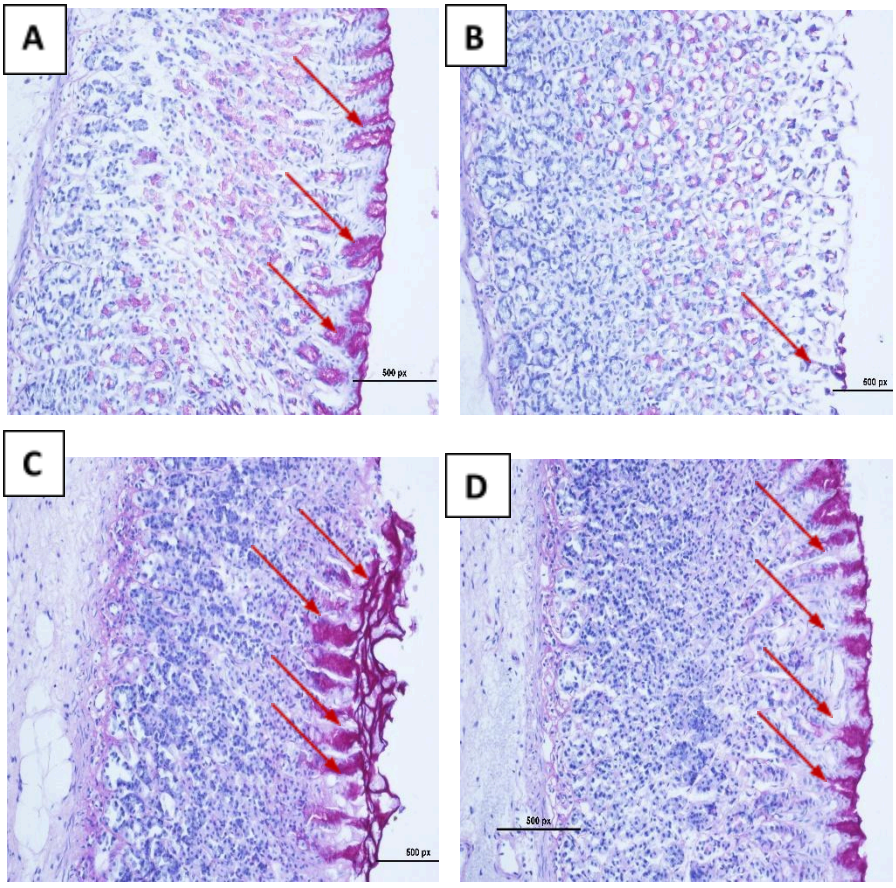


Figure 4.6.1: Impact of *Rheum rhabarbarum* on microscopic perception of the stomach mucosa in rats with ethanol-induced stomach ulcers. (**A**) The normal reference group displayed a normal microscopic assembly of the stomach mucosa. (**B**) The control group with ulcer displayed with

harch structural wounds of the gastric mucosa with swelling and WBC infiltration of the submucous layer (red arrow). (**C**) The group receiving omeprazole demonstrated slight damage to the gastric mucosa. (**D**) The group provided with 250 mg/kg displayed medium injury to the gastric mucosa. (**E**) The group given 500 mg/kg presented rationally minor hurt to the gastric mucosa (H&E stain, magnification $10^{\times}$).

4.6.2 Periodic acid Schiff (PAS) Stain

Experimental rats that were administered *Rheum rhabarbarum* displayed a relatively higher PAS stain concentration of glycoprotein in the epithelium of the stomach (fuchsia color) compared to the control group with ulceration. In other words, the ulcer control group exhibited brutal disruption of stomach cells, swelling, plus an influx of leukocytes. In contrast, treated rats .showed significantly reduced tissue damage and edema as demonstrated by figure 4.6.2



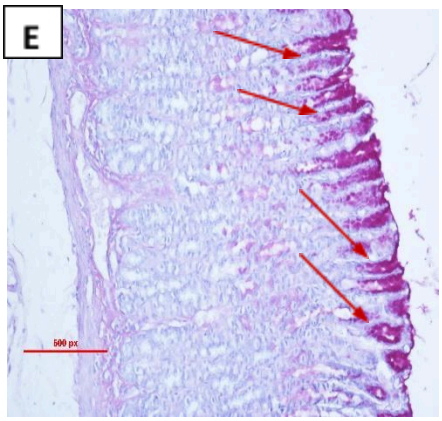


Figure 4.6.2: Impact of Rheum rhabarbarum on the microscopic analysis of the gastric mucosa in rats with ethanol-induced gastric ulcers using PAS glycoprotein staining. (A) Normal group - showing normal gastric mucosal structure with no signs of damage; (B) Ulcer control group - exhibiting severe damage to the gastric mucosa and mild PAS stain (magenta color); (C) Omeprazole group - displaying insignificant damage and intensive PAS staining of the gastric mucosa; (D) Rheum rhabarbarum 250 mg/kg - presenting mild to moderate damage of gastric mucosa with mild PAS stain; (E) Rheum rhabarbarum 500 mg/kg - indicating mild damage of gastric mucosa with moderate PAS stain (PAS stain, magnification 20×).

4.6.3 Immunohistochemistry stain

HSP 70 measurement as well as Bax protein expressions in sections of gastric ulcers tissues was conducted by the mean of Image software.

4.6.3.1 Expression of HSP 70

HSP 70 protein exhibited a decrease in expression in the ulcerated control group and an increase in expression in both the omeprazole and *Rheum rhabarbarum* groups. The HSP 70 protein stain was characterized by a strong brown color-stained antigen in rats that received pre-treatment with either omeprazole or *Rheum rhabarbarum***.** The staining for HSP 70 expression showed an intense brown color in figure 4.6 C, D, and E, indicating a high level of expression. In contrast, the gastric tissue of ulcer control rats in figure 4.6 B exhibited a slightly brown color figure 4.6.3.1.

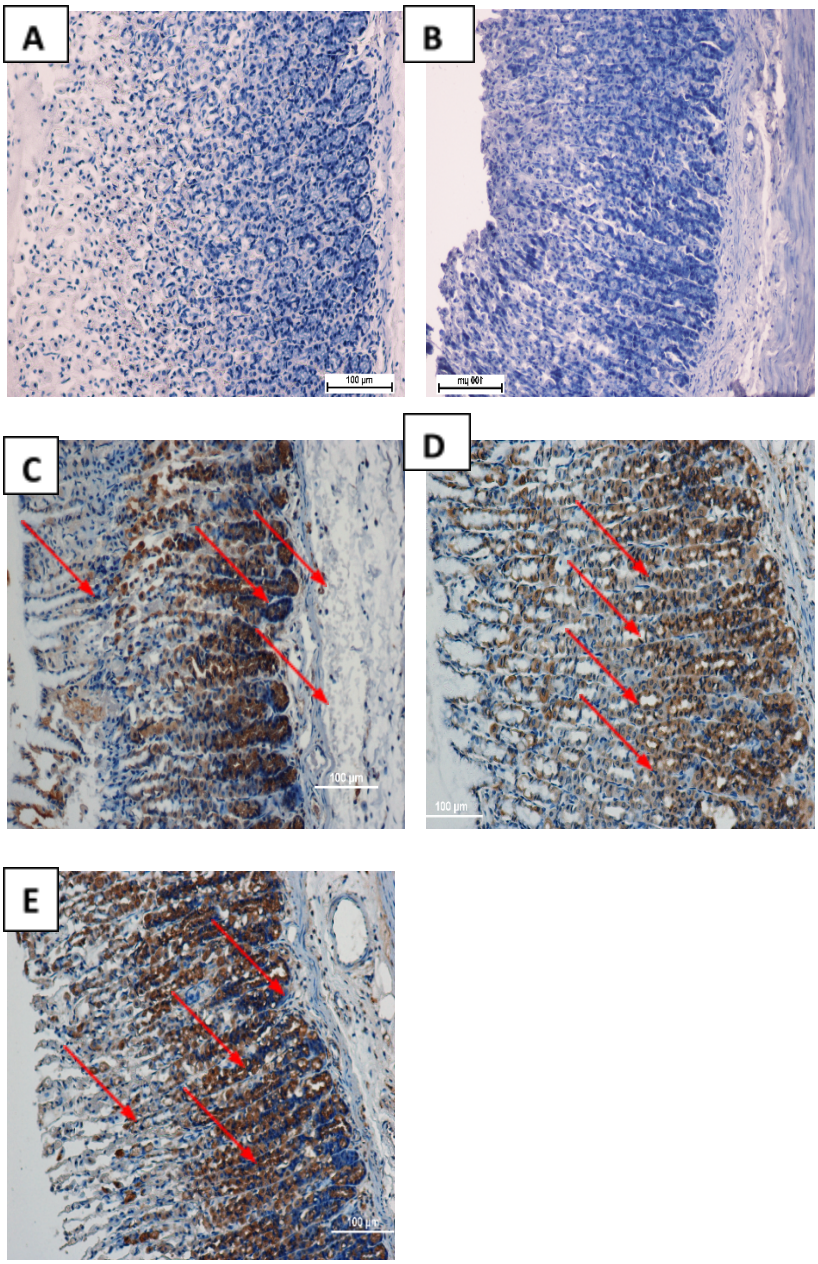
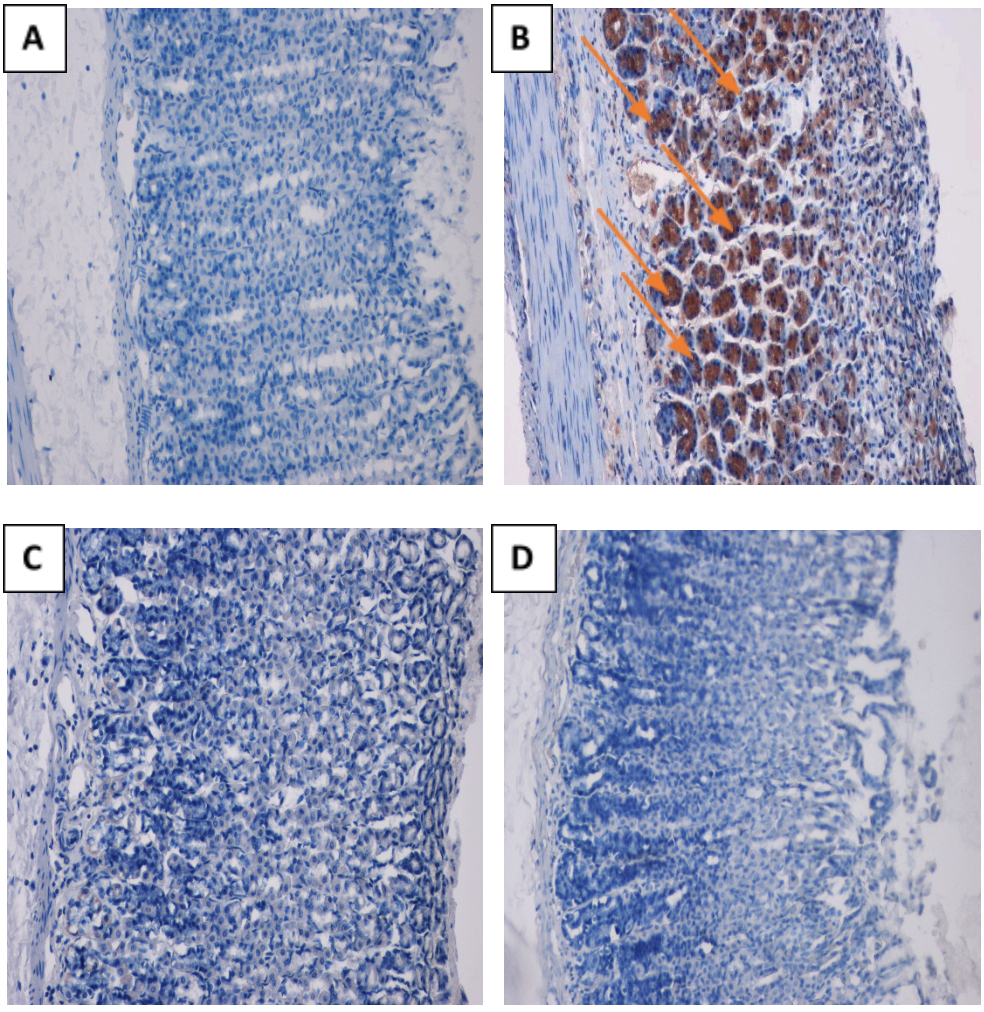


Figure 4.6.3.1: The microscopical observation of HSP-70 protein expression in ethanol-induced gastric ulcers in rats revealed distinct effects. Normal control group displayed normal gastric mucosal structure and very weak expression of HSP 70 protein in the gastric mucosa (**A**). Ulcer control group presented severe injury of the gastric mucosa and down-regulation of HSP 70 protein expression in ethanol-induced gastric ulcers in rats (**B**). Omeprazole group exhibited mild damage of the gastric mucosa and up-regulation of HSP-70 protein expression of gastric mucosa in ethanol-induced gastric ulcers in rats (**C**). *Rheum rhabarbarum* group given 250 mg/kg showed moderate injury of the gastric mucosa and up-regulation of HSP-70 protein expression of the gastric mucosa compared to the ulcer control group in ethanol-induced gastric ulcers in rats (**D**). *Rheum rhabarbarum* group given 500 mg/kg displayed mild damage of the gastric mucosa and up-regulation of HSP 70 protein expression of the gastric mucosa compared to the ulcer control group in ethanol-induced gastric ulcers in rats (**E**). (HSP 70 stain, magnification 20×).

4.6.3.2 Bax Proteins

The expression of the protein Bax exhibited significant up-regulation in the control group exhibiting ulcers and down-regulation in the groups pre-treated with omeprazole and *Rheum rhabarbarum*. Specifically, Bax protein expression was observed to be increased in the control group that is ulcerated and decreased in the groups pre-treated with omeprazole or *Rheum rhabarbarum* as seen in figure 4.6.3.2.



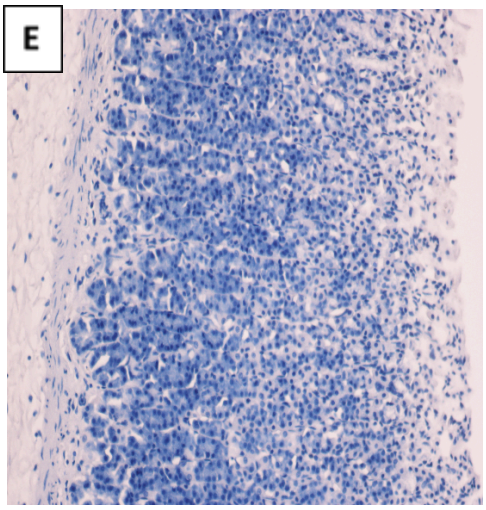


Figure 4.6.3.2: *Effects of Rheum rhabarbarum on the expression of the Bax protein (A–E) in the gastric mucosa during the microscopical observation of rats' gastric ulcers caused by ethanol. The normal control group exhibited a regular gastric mucosal structure with minimal expression of Bax protein in the gastric mucosa (A). In contrast, the ulcer control group displayed severe damage to the gastric mucosa with an increase in Bax protein expression in ethanol-induced gastric ulcers in rats (arrow) (B). The Omeprazole group showed mild damage to the gastric mucosa and a decrease in Bax protein expression compared to the ulcer control group in ethanol-induced gastric ulcers in rats (C). The Rheum rhabarbarum group administered 250 mg/kg demonstrated moderate injury of the gastric mucosa and a decrease in Bax protein expression compared to the ulcer control group in ethanol-induced gastric ulcers in rats (D). Similarly, the Rheum rhabarbarum group given 500 mg/kg exhibited mild damage to the gastric mucosa and a decrease in Bax protein expression compared to the ulcer control group in ethanol-induced gastric ulcers in rats (E). (Bax stain, magnification 20×).*

Effects of *R. rhabarbarum* on Endogenous Antioxidants in Gastric Tissue 4.7 Homogenate Including Superoxide Dismutase (SOD), Catalase (CAT) and (Oxidative Stress Malonedialdehyde (MDA

The analysis of the status of endogenous antioxidant enzymes, specifically SOD and CAT, revealed that the gastric homogenate of ulcer control rats had significantly lower levels of both enzymes compared to those treated with omeprazole and *R. rhabarbarum* extract. Rats treated with omeprazole and *R. rhabarbarum* extracts appeared to have similar levels of SOD stomach homogenate. However, compared to rats treated with omeprazole and *R. rhabarbarum* (500 mg/kg), the amount of CAT stomach homogenate rats treated with *R. rhabarbarum* extract (250 mg/kg) is much lower. On the other hand, the examination of the oxidative stress state revealed

that the quantity of MDA stomach tissue homogenate of ulcer control rats is much higher than those treated with omeprazole and *R. rhabarbarum* extract. MDA levels in rats treated with omeprazole and *R. rhabarbarum* extract high dose (500 mg/kg) appear to be comparable, but in rats treated with low dose *R. rhabarbarum* extract (250 mg/kg), MDA levels are much greater compared to levels documented in rats treated with omeprazole and high dose of *R. rhabarbarum* extract (Table 4.7).

Table 4.7: Impact of *R. rhabarbarum* extract on SOD, CAT, and MDA levels in stomach tissue homogenate during gastric injuries induced by ethanol in rats.

Animal groups	Pre-treatment (5mL/kg)	SOD (U/ml (protein	CAT (nmol/min/ml (protein	MDA (umol/L)
G1 Negative control	CMC 0.5%	0.57	117	2.5
G2 Ulcer control	CMC 0.5%	0.26	60	16
G3 Omeprazole	mg/kg 20	1.04	135	3.5
G4 *R. rhabarbarum*	mg/kg 250	0.75	109	5.5
G5 *R. rhabarbarum*	mg/kg 500	0.91	125	4.03

Impact of *R. rhabarbarum* Extract on The Quantity of Cytokines 4.8

Rats given omeprazole and *R. rhabarbarum* extract had meaningfully diminished levels of TNF-a, and IL-6 in gastric homogenate, according to the results of the current experiment. However, compared to ulcer control (G2), the level of IL-10 in the gastric juice mixture of rats .administered omeprazole and *R. rhabarbarum* isolate is much higher

Table 4.8: Effect of *R. rhabarbarum* extract on TNF a, IL 6 and IL10 following gastric ulcer .induced by ethanol in rats

Animals group	**Pre-treatment**	TNF-α **(Pg/ml)**	**IL- 6 (Pg/ml)**	**IL-10 (Pg/ml)**
G1 Negative control	0.5% CMC	98	146	246
G2 Ulcer control	0.5% CMC	530	345	107
G3 Omeprazole	20 mg/kg	128	137	238
G4 *R. rhabarbarum*	250 mg/kg	244	179	186
G5 *R. rhabarbarum*	500 mg/kg	131	149	234

Chapter V

Discussion .5

The historical trajectory of medicine, spanning from prehistoric times to the present day, highlights an enduring link between the utilization of plants and the management of peptic ulcers. Despite the significant strides made in the scientific understanding of peptic ulcers, the supplementary use of plants and their derivatives persists. Plant-based therapies, exemplified by the enduring utilization of rhubarb, have been instrumental in promoting digestive health. *Rheum rhabarbarum*, with its multifaceted attributes including anti-inflammatory properties, enhancement of mucus production, and antioxidative effects, emerges as a promising adjunct for addressing a spectrum of gastrointestinal disorders, ranging from gastritis to peptic ulcers. The exploration of its therapeutic potential is, however, accompanied by a prudent consideration of its potential for toxicity. For this reason the objective of this study was to aim to conduct a comprehensive evaluation of *Rheum rhabarbarum*, encompassing various aspects. Firstly, the investigation focuses on determining the acute toxicity of Rheum rhabarbarum through a series of systematic tests. Secondly, the anti-ulcerogenic potential of *Rheum rhabarbarum* extracts is macroscopically assessed, particularly concerning absolute ethanol-induced gastric damage in rats.

In term of kidney biomarkers, rats receiving *Rheum rhabarbarum* extract were having approximately the same levels of sodium, potassium, chloride, urea and creatinine as the rats receiving only a vehicle regardless of the extract dose and the gender of the rats. Moreover, in terms of liver biomarkers, total protein, Albumin, ALP, ALT and AST were nearly identical between rats receiving only the vehicle and rats receiving low and high dose of *Rheum rhabarbarum* extract. These results reveals that the administration of *Rheum rhabarbarum* extract, at both low and high doses, did not result in notable alterations in kidney and liver function biomarkers. This is a positive outcome, suggesting that the extract may not have adverse effects on these crucial organs under the conditions tested. Moreover, our finding are evidence through the historical use of *R. rhabarbarum*. In Western culinary practices, the stalk of rhubarb

stands out as a versatile ingredient, renowned for its rich content of dietary fiber, anthocyanins, and the flavonol quercetin and its derivatives. Typically consumed raw as a vegetable, it finds its way into various culinary delights such as wine, jam, and juice. Through the addition of sugar and heat, the inherently sour taste of the stalk transforms into a delightful sweetness, making it a popular choice for pies and tarts. This has earned rhubarb the affectionate moniker of a "pie vegetable. However, only roots and stalk were used that since the rhubarb leaf contains a substantial amount of oxalic acid and is not suitable for consumption (Püssa, et al., 2009).

In addition, the administration of absolute ethanol to induce peptic ulcers in rats had led to significant damage in the stomach, resulting in severe gastric lesions. The ulcer control group exhibits extensive hemorrhagic lesions on the stomach mucosa. In contrast, the experimental groups treated with *Rheum rhabarbarum* exhibited a significant reduction in gastric mucosal ulceration, comparable to the Omeprazole-treated group, which showed minor injuries to the gastric mucosal surface. Similar to our findings, a study conducted to look at how Rhubarb powder's anti-inflammatory qualities can protect against stomach mucosal damage brought on by an acute exposure to ethanol revealed that the stomach of rats used as ulcer control, exhibited significant histopathological changes, including focal necrosis of the gastric mucosa, infiltration of inflammatory cells in the mucosa, and submucosal edema accompanied by inflammatory cell infiltration. However, a pronounced ameliorative effect was observed in the gastric tissue of rats from the other groups, including those fed an experimental diet containing 5% Rhubarb powder and 10% Rhubarb powder (Awaad, 2022).

Furthermore, the results of this study demonstrate a noteworthy increase in mucus weight among rats administered with the extract component of *R. rhabarbarum* when contrasted with the ulcer control group (G2). Notably, ethanol treatment led to a significant reduction in mucus secretion in ulcer control rats, resulting in markedly lower mucus weight compared to the reference group marked as (G1), group 3 (G3) that received omeprazole, as well as (G4) where they received250 mg/kg) and lastly (G5) who received 500 mg/kg of *R. rhabarbarum*. These findings were demonstrated by an investigation assessing the hepatoprotective impact of rhubarb extract in a mouse model simulating binge drinking while understanding the role of the gut microbiota in influencing the associated metabolic effects. Nevertheless, it was been found that Rhubarb stimulates the digestive mucosa's goblet cell proliferation, leading to increased production of

mucus. This enhanced mucus secretion serves to strengthen the intestinal mucosal barrier by forming a protective mucosal layer (Neyrinck, et al., 2017).

Gastric mucus pH value assessments in the rat experimentations reveal that pretreatment by mean of omeprazole (G3) and the above ground part concentrate of *R. rhabarbarum* (250 mg/kg for group number 4 and 500 mg/kg for group number 5) significantly lessened sores formation and resulted in lower stomach acidity. Consequently, the gastric mucus pH of the in G3, 4, and 5 was significantly higher than that in the ulcer control group (G2). The pH levels of G 5 as well as 3 appear to be near enough to be said similar. Yet, the extract's effect seems to be dose-dependent. A study carried out to determine whether alterations in the pH of the digestive tract may affect the bioaccessibility of oxalate in food that was prepared both with and without a high calcium content and had high oxalates revealed that ollowing the addition of samples, the stomach medium exhibited an average pH shift from 1.07 to 2.0 (Nguyen & Savage, 2020). Therefore supporting our finding that *R. rhabarbarum* decrease the gastric acidity.

The control group with ulceration exhibited extensive damage to the epithelial layer of the stomach, characterized by profound abrasions in the mucous membrane accompanied by swelling and white blood cell inflow in the subcutaneous layer. In contrast, test group rodents that were administered *R. rhabarbarum* demonstrated enhanced protection of the gastric epithelium, evident by a decrease in the ulcerated area. Additionally, there was a relief in swelling and inflammatory compartment infiltration within the submucosa in these experimental groups. Correspondingly, a similar study revealed that rats treated with low dose implied a slight submucosal edema while histological structure of rats treated with high dose appeared normal (Awaad, 2022). Confirming that the improvement was dose dependent.

The heightened intensity of PAS staining for glycoprotein (magenta color) observed in the gastric epithelium of experimental rodents fed with *Rheum rhabarbarum*, as opposed to the ulcerated control group, suggests an increase in the presence of glycoproteins in the gastric mucosa. This difference in staining intensity may indicate that *Rheum rhabarbarum* has a positive influence on glycoprotein production or secretion in the gastric epithelium. Glycoproteins play a role in mucosal protection and could contribute to the observed defense of the gastric epithelium in the experimental group, potentially explaining the reduced ulceration in comparison to the control group. A recent paper examining the mechanism in which the Tangshen formula that contains

Rheum rhabarbarum has anti-inflammatory benefits on Sprague Dawley rats demonstrated a higher PAS staining in the experimental group (Du, et al., 2018).

The ulcerated control group exhibited a decrease in the expression of the HSP 70 protein and an increase in the expression of the Bax protein. Conversely, the groups pre-treated with omeprazole or *Rheum rhabarbarum* showed an increased expression of heat shock protein 70.and a down- alteration of Bax proteins. The staining for HSP 70 protein, represented by a strong brown color, was notably intensified in rats that received pre-treatment with omeprazole or *Rheum rhabarbarum*. This suggests that omeprazole and *Rheum rhabarbarum* may contribute to an increase in HSP 70 expression, potentially indicating a protective response against ulceration (Shichijo, et al., 2003). Similarly, the observed down-regulation of Bax protein in these pre-treated groups suggests a potential anti-apoptotic effect, contributing to the protective response against ulcer formation (Arab, et al., 2015). One study experimenting the regulation of HSPs by TCM revealed that *Rheum rhabarbarum* increases HSP70 levels while decreasing Bax levels (Wang, et al., 2022). These results were consisting with our finding.

The assessment of endogenous antioxidant enzymes, specifically SOD and CAT, revealed a notable decline in their levels in the gastric tissue extract from rats with ulcers when compared to animals infused with omeprazole and *R. rhabarbarum* extract. The levels of SOD in the stomach homogenate of rats treated with omeprazole and *R. rhabarbarum* extracts appeared to be equivalent. However, the CAT levels in the stomach homogenate of rats treated with *R. rhabarbarum* extract (250 mg/kg) were drastically lessened than those in rats managed using omeprazole and *R. rhabarbarum* (500 mg/kg). The observed decrease in the levels of antioxidant enzymes produced endogenously, SOD and CAT, in the gastric homogenate of ulcer reference rats may be indicative of heightened oxidative stress in the gastric tissues of these rats. Oxidative stress often leads to the depletion of antioxidant defenses, resulting in reduced SOD and CAT activity. The similarity in SOD levels between rats treated with omeprazole and *R. rhabarbarum* extracts suggests a comparable antioxidant effect from both treatments. This similarity might imply that *R. rhabarbarum* extract, contributes to the preservation or restoration of SOD levels, potentially mitigating oxidative damage.On the other hand, the significantly lower CAT levels in subjects in the experimental set treated with *R. rhabarbarum* extract (250 mg/kg) considered with those remedied with omeprazole besides higher dose of R. rhabarbarum (500 mg/kg) may

indicate a dose-dependent effect. The lower CAT levels at the lower dosage of R. rhabarbarum extract could suggest a less pronounced impact on catalase activity, possibly due to a threshold effect where a higher dose is needed for a more substantial antioxidant response. However, further investigation would be necessary to elucidate the precise mechanisms and dose-dependency of these antioxidant effects. Moreover, the analysis of oxidative stress status revealed a notable increase in the quantity of MDA in stomach tissue homogenate of ailment reference rats labeled as G2 juxtaposed to those remedied with omeprazole and *Rheum rhabarbarum* draw out. This indicates that ulcer control rats experienced higher levels of lipid peroxidation, as reflected by elevated MDA levels, suggesting oxidative damage to cellular membranes. In contrast, the lower MDA levels in rats treated with omeprazole and *R. rhabarbarum* extract imply a potential antioxidative effect, as these treatments appeared to mitigate lipid peroxidation, contributing to a more favorable oxidative stress profile in the stomach tissue. Similarly, a recent research revealed resuls identical to our finding where Rheum exhibited a substantial reduction in both the ulcer index and the level of MDA in the mucosa of stressed rats. Furthermore, it elevated the activity of SOD as well as CAT in both serum and gastric mucosa. Notably, Rheum demonstrated remarkable protective effects against experimentally induced gastric ulcers in rats (Awaad, 2022).

Lastly, the results from this experiment regarding the impact of *R. rhabarbarum* on plasma cytokine profile demonstrate that rodent species treated with omeprazole and *R. rhabarbarum* draw out exhibited notably reduced levels of TNF-α and interleukine number six in gastric homogenate in comparison to those in ulcer reference rats. This reduction suggests a potential comparable anti-inflammatory effect of *R. rhabarbarum* extract and omeprazole, as elevated TNF-α and IL-6 levels are often associated with inflammatory processes. Conversely, the IL-10 levels in gastric homogenates of rats fed with omeprazole and *R. rhabarbarum* extract were significantly lower compared to those in the ulcer control group. This decrease in IL-10, known for its anti-inflammatory properties, might seem counterintuitive. However, it's essential to note that reduced IL-10 levels could be interpreted as a modulation of the immune response, potentially contributing to the observed anti-ulcer and anti-inflammatory effects in these experimental groups. In a clinical examination that was controlled by randomization, with more than 100 patients undergoing appendix removal surgically, the application of a 1 mg/g ointment containing *R. rhabarbarum* twice daily demonstrated significant efficacy. This treatment resulted

in a notable reduction in inflammation and improvement in the healing process of sutures (Li, et al., 2016). On the other hand, an in vitro screen of various medicinal plant extracts revealed that the aqueous extract derived from *R. rhabarbarum* displayed the ability to inhibit the tumor necrosis factor α. Additionally, it demonstrated a suppressive effect on the illustration of adhesive particles, specifically VCAM-1 and ICAM-1, as well as the chemoattractant protein-1 of monocyte (MCP-1) and interleukins including interleukin 1 and 6. Moreover, the inhibition observed in the suppression of tumor necrosis factor α, along with the downregulation of adhesion molecules and monocyte chemoattractant protein-1, seems to follow a dose-dependent pattern with the extract from *R. rhabarbarum* that is liquid-based (Moon, et al., 2006). Therefore, this results were parallel to our finding, highliting the anti-inflammatory and antioxidant effect of Rhubarb.

This study has some limitation including the absence of standardized *Rheum rhabarbarum* extracts with defined concentrations of active compounds, which could potentially impact the consistency of therapeutic outcomes. Additionally, the scope of this study is confined to the short-term safety assessments of *Rheum rhabarbarum* extract. Long-term safety remains unexplored, necessitating extended investigations to ascertain whether repeated administration might induce adverse effects or toxicity over an extended duration. The study's findings should be interpreted with consideration of this temporal limitation.

Expanding research from animal studies to clinical trials represents a pivotal step in gauging the translational applicability of Rheum rhabarbarum extract in human subjects. Conducting clinical trials with individuals experiencing gastric issues holds promise for yielding significant insights into the extract's effectiveness and safety under real-world conditions. This transition from preclinical studies to human trials opens avenues for further investigation, offering an opportunity to validate the extract's therapeutic potential and advance its application in clinical settings.

Chapter VI

Conclusion and Recommendations .6

Conclusion 6.1

The findings of this study indicate promising outcomes regarding the potential benefits of *Rheum rhabarbarum* extract in mitigating gastric damage and promoting gastroprotection. The administration of both low and high doses of the extract resulted in a notable decrease in intragastric acidity when compared to the ulcer control group. Additionally, the extract exhibited a level of ulcer inhibition comparable to the positive control (omeprazole), with the high dose demonstrating superior inhibitory effects. Microscopic examination revealed improvements in stomach epithelium defense and a reduction in ulcerated areas in rats treated with *Rheum rhabarbarum*, as evidenced by H&E and PAS staining.

Furthermore, the study observed alterations in protein expressions associated with gastric health. The decrease in the manifestation of HSP 70 protein in the ulcerated reference group, contrasted with increased expression in groups treated with omeprazole and *Rheum rhabarbarum*, suggests a potential protective effect of the extract. Similarly, the increase in Bax protein expression in the ulcerated control group, mitigated in groups pre-treated with *Rheum rhabarbarum*, aligns with the extract's potential anti-apoptotic properties.

Biochemically, the extract demonstrated antioxidant effects, as seen in elevated degrees of CAT and SOD in gastric homogenates paralleled to the ulcer control team. Additionally, the observed TNF-α and IL-6, two pro-inflammatory markers, reduction with the increase in IL-10, that represent an anti-inflammatory marker, further support the anti-ulcerogenic potential of *Rheum rhabarbarum* extract.

In summary, this comprehensive evaluation underscores the multi-faceted positive impact of *Rheum rhabarbarum* extract on biochemical and histological parameters related to gastric health, suggesting its potential as a therapeutic agent in countering gastric damage.

Recommendations 6.2

1. Since the high dose of Rheum rhabarbarum extract demonstrated superior inhibitory effects on gastric ulcers, it would be valuable to conduct further studies to optimize the dosage. This can help determine the most effective and safe dosage for potential therapeutic applications.
2. Further plant extract from other countries or regions are recommended to be tested.
3. Given the promising anti-ulcerogenic effects observed, it is advisable to conduct long-term safety assessments of Rheum rhabarbarum extract. This would involve extended studies to ensure that repeated administration does not lead to adverse effects or toxicity over an extended period.
4. Exploring the potential synergistic effects of Rheum rhabarbarum extract with conventional anti-ulcer medications, like omeprazole, could be explored. This combination therapy may enhance therapeutic outcomes while potentially reducing the required dosage of conventional medications, minimizing side effects.
5. Statistical analysis are also recommended in order to assess the presence of any significance.

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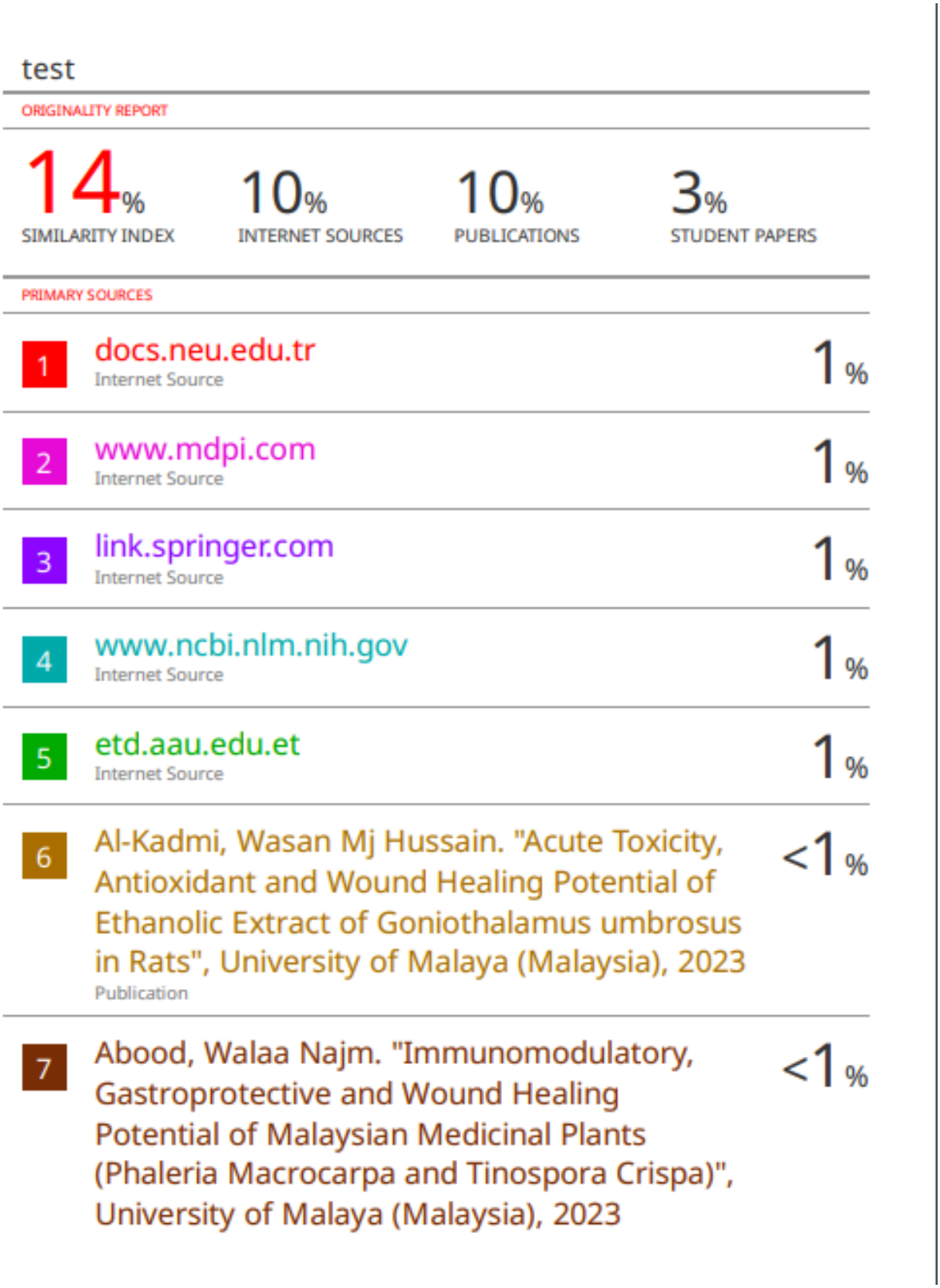
Appendixes

Appendix 1



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Halo Hameed

Microbiologist

Dedicated professional with a solid educational foundation, holding a Bachelor's in Biology and a Master's in Microbiology. With a proven track record as a medical assistant at Soran Private Hospital, I have honed my skills in patient care and organizational efficiency. My background extends to medical laboratory environments, where I am adept at navigating laboratory procedures and utilizing analytical techniques. Armed with a passion for microbiology and a commitment to excellence, I bring a blend of academic knowledge and practical experience, positioning me as a valuable asset in healthcare and laboratory settings.

Contact

Phone
+964 750 747 0053

Email
halonawzad774@gmail.com

Address
Erbil, Iraq

Skills

- Fast Learner
- Dedicated Team Player
- Computer Skills
- Microsoft Office Word
- Microsoft Powerpoint
- Microsoft Excel
- Flexibility and Adaptability
- Laboratory Techniques
- Laboratory Quality Control
- Medical Billing

Language

Kurdish - Native
English - Fluent
Turkish - Fluent
Arabic - Fluent

Experience

June 2019 - Oct 2019
Soran Private Hospital, Erbil

Medical Assistant

During my tenure as a medical assistant at Soran Private Hospital in 2019, I gained invaluable practical experience in a healthcare setting. Assisting healthcare professionals in various medical procedures, managing patient appointments, and maintaining accurate records honed my clinical and organizational skills. The fast-paced environment of Soran Private Hospital allowed me to thrive under pressure while ensuring the delivery of quality patient care. Interacting with diverse patients enhanced my communication and interpersonal abilities, fostering a compassionate approach to healthcare. This experience at Soran Private Hospital not only solidified my understanding of medical practices but also contributed significantly to my professional growth in the dynamic field of healthcare.

May 2020 - Oct 2020
Soran Private Hospital, Erbil

Medical Assistant

In my role as a medical assistant at Soran Private Hospital, I've gained diverse experience, from foundational tasks to active participation in medical procedures. My responsibilities extended to patient communication, medical billing processes, and leadership in training new assistants. This experience has honed my organizational skills, deepened my commitment to patient-centered care, and equipped me with a versatile skill set to thrive in the dynamic field of healthcare.

Education

Sep 2016 - June 2020
Cihan University, Erbil

Bachelor of Science in Biology

Feb 2021 - Jan 2024
Near East University, Nicosia

Masters in Microbiology

Graduated with a 3.17 CGPA

Cihan University-Erbil
College of Science

Date: 21/12/2023

RESEARCH ETHICS COMMITTEE APPROVAL SHEET

Title of the project: Gastroprotective Effects of Rheum Rhabarbarum Extract against Ethanol-Induced Gastric Ulcer Disease in Cihan University, Erbil, Iraq: in Vivo Study

Principle investigator: Assistant Professor Dr. Esref Celik
Co-investigators: Assistant Professor Dr. Ahmed Salem

Cihan University - Erbil
College Of Science
Department of Medical Microbiology

Head of Committee

Committee Member

A.P.Dr Muhsin Alghezzi
21/12/2023

Committee Member

Prof Dr. Mahmood Ameen