TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

EFFECT OF HYPERICUM PERFORATUM & PUNICA GRANATUM ON GASTRIC ULCER IN RAT

MOHAMMED M.HUSSEIN M.RAOUF AL-SALIMAGHA

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

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DECLARATION

Hereby, I declare that this thesis study is my own study, I had no unethical behaviors in all stages from planning of the thesis until writing there for, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list, and had no behavior of breeching patent rights and copyright infringement during the study and writing of this thesis

Mohammed M.Hussein M.Raouf AL-SALIMAGHA

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DEDICATION

THIS THESIS IS DEDICATED TO:

MY SUPERVISORS

MY BELOVED PARENTS

MY DEAR BROTHERS

MY FRIENDS

ALL WHO HELPED ME

LIST OF CONTENT

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

MALT	mucosa-associated lymphoid tissue
HP	Helicobacter pylori
PUD	Peptic Ulcer Disease
NSADs	Non-steroidal anti-inflammatory drugs
GIS	Gastrointestinal System
SJW	St. johns wort
WHO	World Health Organization
GERD	Gastro-esophageal reflux disease
SD	Sprague Dawley
pH	potential of hydrogen
cagA	cytotoxin-associated gene A
vacA	Vacuolating cytotoxin A
dupA	Duodenal ulcer promoting gene A
PAI	Pathogenicity Island
TPMs	Tyrosine phosphorylation motifs
MXCHT	Modified Xiaochaihutang

HPLC	High-performance liquid chromatography
GML	Gastric MALT lymphoma
RUT	Rapid Urease Test
FISH	Fluorescence in situ hybridization
NPV	Net Present Value
HDDT	High-dose dual therapy
IBV	Infectious bronchitis virus
HPE	Hypericum perforatum extract
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphate
LDH	High Density Lipoprotein
NAFLD	Non-alcoholic fatty liver disease
QSHYD	Qushi Huayu Decoction
TG	Triglyceride
UV	Ultraviolet
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
MEPP	Methanolic Extract Pumegranate Peel
PPE	Pumegranate Peel Extract
PE	Pumegranate Extract
AST	Aspartate Aminotransferase

ESGD	Equine squamous gastric disease
OME	Omeprazole
CFU	Colony-forming unit
BHI	Brain Heart Infusion
RPM	Revolutions Per Minute
MM	Millimeter
UA	Ulcer Area
MDA	Malondialdehyde
AMP	Adenosine monophosphate

ABSTRACT

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Gastric ulcer is a chronic condition that occurs when the mucosa of the stomach is broken. There is a physiological equilibrium between aggressive factors and mucosal defense. This study aimed to determine the prevention level and efficiency of two herbal medicinal plants (*Hypericum perforatum* and *Punica granatum*) and compare them with the omeprazole drug.

Many groups were prepared from Albino male rats, first control group (inoculate with *H. pylori* and fed with standard pellet), Second group, rats inoculated by *Helicobacter pylori* (*H. pylori*) and prevented with aqueous extract *H. perforatum* in two dosages (250mg/kg, 500mg/kg), Third group, rats inoculated by *H. pylori* and prevented with aqueous extract *P. granatum* in two dosages (250mg/kg, 500mg/kg), and last group inoculated by *H. pylori* and prevented with standard drug omeprazole at the dose (20mg/kg).

The result showed that *H. perforatum* inhibits (50.65%) stomach ulcer formation with a high dose. *Punica granatum* inhibits (84.60%) stomach ulcer formation with a high dose. Omeprazole's' group results showed (24.50%) stomachs ulcer formation.

Although the result of the current study improves, a high dosage of aqueous extracts of plants has more effectiveness than the low dosage of aqueous extracts of plants.

Keywords: Gastric ulcer, *Helicobacter pylori*, antibacterial activity, gastrointestinal tract, peptic ulcer

ÖZET

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Mide ülseri, mide mukozası kırıldığında ortaya çıkan kronik bir durumdur. Agresif faktörler ve mukozal savunma arasında fizyolojik bir denge vardır. Bu çalışmada, iki şifalı bitkinin (*Hypericum perforatum* ve *Punica granatum*) önleme düzeyi ve etkinliğinin belirlenmesi ve omeprazol ilacı ile karşılaştırılması amaçlanmıştır.

Albino erkek sıçanlardan birçok grup hazırlandı, birinci kontrol grubu *(H. pylori* ile aşılayın ve standart pelet ile beslenen), İkinci grup, Helicobacter pylori (*H. pylori*) ile aşılanan sıçanlar ve iki dozajda sulu ekstrakt H. perforatum ile önlendi (250mg / kg, 500mg / kg), Üçüncü grup, H. pylori ile aşılanmış ve sulu ekstrakt *P. granatum* ile iki dozajda (250mg / kg, 500mg / kg) önlenmiş ve son grup *H. pylori* ile aşılanmış ve önlenmiştir. standart ilaç omeprazol dozunda (20 mg / kg).

Sonuç, *H. perforatum'un* yüksek dozda mide ülseri oluşumunu (% 50.65) engellediğini gösterdi. *Punica granatum* yüksek dozda mide ülseri oluşumunu (% 84.60) inhibe eder. Omeprazole'ün grup sonuçları (% 24.50) midede ülser oluşumu gösterdi.

Mevcut çalışmanın sonucu iyileşse de, bitkilerin yüksek dozajlı sulu özleri, bitkilerin sulu özlerinin düşük dozajından daha etkilidir.

Anahtar Kelimeler: Mide ülseri, *Helicobacter pylori*, antibakteriyel aktivite, gastrointestinal sistem, peptik ülser

1 INTRODUCTION

1.1 *Helicobacter pylori*

Helicobacter pylori (H .pylori) is a highly motile, spiral-shaped and gram negative bacterium that colonies the intestine of 50-80% of individuals around the world (Palamides et al., 2020). "*H .pylori*, usually in the form of gastritis, peptic ulcer disease, or also gastric malignancy, is a bacterium that causes damage to the gastric mucosa. The most frequent clinical manifestation of *H. pylori* infection is gastritis". "The development of inflammation of the sub-mucosa or gastric mucosa because of *H. pylori* induces histological modifications that are mild-to-severe". The extent of gastritis is correlated with the density of *H. pylori*, damage to the gastric mucosa and inflammation (Yulizal et al., 2020).

During early infancy, it is acquired and typically continues throughout life. Prevalence varies between developed and developing countries and is caused by a variety of factors, such as age, genetic predisposition of the host, sanitation, nutritional and socioeconomic factors. Although the infection appears asymptomatic in most affected patients, "it can lead to gastritis, peptic ulcer disease and is also a risk factor for the growth of gastric cancer and MALT lymphoma" (Palamides et al., 2020).

H. pylori affected individuals can be evaluated on the basis of the degree and duration of mucosal disruption and atrophy known as metaplastic epithelia with or without intraepithelial neoplasm or dysplasia. Integrative experiments and human studies have demonstrated that the eradication of *H. pylori* may reduce the risk of gastric cancer, which is related to the degree of genetic alterations and epigenetic changes present at the time of eradication of *H. pylori* (Liou et al., 2020).

The aggregate prevalence of infection is associated with socio-economic factors. The incidence of middle-aged people in many developed countries is more than 80 per cent, compared to 20 per cent to 50 per cent in advanced countries. The mechanism of

infection is caused by the ingestion of bacteria through the mouth and is particularly infectious in child families. In developing nations, bacteria propagate directly from person to person by vomiting or spit. Other infectious channels such as drinking water are popular in developed countries. Actually, *H. pylori* infection has no evidence of animal-to-human transmission, but *H. pylori* is present in primates other than humans, and often in animals other than humans (Hoi, H 2020).

Over the past couple of years the amount of peer-reviewed *H. pylori* publications increased rapidly, from less than 200 in 1990 to about 1.500 annually. While important issues such as *H. pylori* transmission route are not yet known. While the prevalence of *H. pylori* decreases in the western world, the colonization of *H. pylori* gastricity in the developing world remains widespread. A variety of methods can be used to diagnose *H. pylori* infection and also antibiotic can be used as a treatment. Unfortunately, an increase in antibiotic resistance is beginning to affect the effectiveness of therapy and despite *H. pylori* effects, preventive vaccination do not yet exist. A better understanding of *H. pylori* the persistence and pathogenesis is therefore needed to help develop new interventions and preventative strategies (Kusters et al., 2006).

In the etiology of a variety of gastro-duodenal diseases *Helicobacter pylori* (*H. pylori*) plays a pivotal role, and the elimination of infection is known to alter the course of the peptic ulcer by encouraging ulcer healing, decreasing the rate of ulcer recurrence and its complications. *H. pylori* has consistently been shown to be over 90 percent in duodenal ulcers (DU) and over 80 percent in non-NSAID (non-steroidal anti-inflammatory drugs) mediated gastric ulcers (GU) in recent years with a higher sample size and better experience with diagnoses of *H. pylori* infection. In contrast, a decreasing *H. pylori* contamination amount amongst peptic ulcer patients has been detected elsewhere. In a earlier study by (Kang et al., 1990) about a decade ago on Singaporean patients, the *H. pylori* contamination amount was identified only in 66% of patients with GU, 86% of patients with DU and 75% with collective GU & DU. As the current trend of *H. pylori* prevalence in our local peptic ulcer population has not been retraced, we aimed to looking back determine the recent prevalence of *H. pylori* amongst peptic ulcer patients in a hospital location (Vu & Ng, 2000).

The epidemiology of *H. pylori* has changed over the past decades through weakening of pollution outbreaks in most countries. The changing dynamics of bacteria are associated with similar reductions in peptic ulcer disease and gastric cancer, and may affect the changing dynamics of other diseases such as gastro esophageal reflux disease, allergies, and asthma. Last year, several studies reported data on the prevalence of *H. pylori* infection in both adults and children in Europe, Canada, Latin America, Asia and Africa (Taylor and Blaser, 1991).

Since the discovery of this pathogen in 1982, various tests have been described to detect *H. pylori* infection. There have been no recent breakthroughs on this subject, but numerous original articles, especially from emerging countries, were published last year on other molecules. Non-molecular diagnostic tests for *H. pylori* suggest that careful methodology is required to obtain reliable results, and that change in methodology or lack of local validation can have a strong negative impact on the reliability of the test.

H. pylori recommended that if the exponential endoscopy result is negative for acute upper gastrointestinal bleeding, delayed testing should be performed using histology or urea breathing test (UBT) 4-8 weeks after the onset of bleeding. Finally, the 2nd Asia-Pacific Consensus Guidelines for *H. pylori* infection recommend UBT as the most accurate non-invasive test, but local validation is not possible because the manufacturer has introduced a number of changes, including reduction in urea dose or pre-elimination of citric acid. Required management that can affect the reliability of the test. In addition, the consensus group considers stool testing to be acceptable and suggests that serology plays a limited role in the diagnosis of *H. pylori* (Bessède et al., 2017).

H. pylori infection is associated with peptic ulcer disease in children. Infections often occur in childhood and appear as chronic infections throughout life unless treated. Accordingly, early eradication of *H. pylori* has become an important issue.

Gradually increasing antibiotic resistance is a major problem in *H. pylori* eradication worldwide. Metronidazole and levofloxacin were above 15%, a common threshold for choosing an alternative empirical therapy. In the same study, the joint prevalence of primary resistance to clarithromycin, metronidazole and levofloxacin was 26%, 31% and 15% in Taiwan, respectively. Clarithromycin resistance in children has been increasing in the last 20 years and has now reached 23.4% in Taiwan. As you know, the successful

eradication of *H. pylori* depends on the treatment regimen and antimicrobial susceptibility. Therefore, for the eradication of *H. pylori*, an appropriate first-line treatment regimen to overcome the effects of antibiotic resistance is essential (Su et al., 2021).

H. pylori contamination is an independent risk factor for peptic ulcer disease, and previous studies have shown that 43 to 56% of people with peptic ulcer bleeding (PUB) are infected with *H. pylori*. *H. pylori* eradication can significantly reduce the risk of peptic ulcer and recurrent ulcer bleeding, so *H. pylori* treatment is recommended for all patients infected with peptic ulcer. In addition, testing and treatment strategies for *H. pylori* should also be considered in high-risk patients with a previous history of upper gastrointestinal bleeding (UGIB) or ulcers, or a history of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs). (Guo et al.,2021).

(*H. pylori*) are a major etiology of chronic gastritis, peptic ulcer, gastric cancer, and lymphoid tissue lymphoma associated with the gastric mucosa. Although triple therapy, consisting of two antibiotics and a proton pump inhibitor, shows a high eradication rate, the rate of resistance to antibiotics is increasing now. In addition, undesirable side effects such as nausea, vomiting, epigastria pain, abdominal discomfort, and diarrhea are often unavoidable. Therefore, it is of utmost importance to find a non-antibiotic that is effective and has no side effects. Many medicinal plants have been reported to have antibacterial action against *H. pylori*. However, there are few detailed studies on the antibacterial mechanism. Hydrophobic interactions generally appear to be involved in prokaryotic and eukaryotic cell interactions. They play an important role in the physicochemical and biological behavior of various types of organic compounds. Attachment of pathogenic bacteria to host cells is required in many Gram-negative intestinal pathogenic bacteria-induced infections and can be influenced by the surface hydrophobicity of microbial cells (Voravuthikunchai et al., 2006).

1.2 Peptic Ulcer

Peptic Ulcer The most frequent ulcer in a region of the gastrointestinal tract is peptic ulcer, also known as peptic ulcer disease (PUD) or Peptic ulcers occur globally, and gastric cancer is the second most frequent cause of death from malignant diseases. It is classified as mucosal erosions that are equal to or greater than 0.5 cm. Although most ulcers are

infected with *H. pylori*, a spiral-shaped bacterium dwelling in the acidic atmosphere of the stomach (Moghaddam et al., 2013). Majority in *H. pylori* infection occurs in developed countries where up to 80 per cent of middle-aged people may be affected. In the other hand, the average world prevalence of *H. pylori* Infection with pylori was reported to be 58%. While most people are afflicted with *H. pylori* remain asymptomatic throughout their lifespan, and basically all experience systemic inflammation (Wang et al., 2013).

To the ulcer, the *H. pylori* bacterium contributed. It induces chronic, infectious diseases that control the stomach's functioning and structure. A major source of universal death and morbidity was the virus. In contrast, ulcers have been exacerbated by the long-term application of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs). If the *H. pylori* test is positive, some antibiotics, such as Amoxicillin and Tetracycline, can be administered alongside other medications that reduce stomach acidity, such as proton pump inhibitors, in various forms (Ragab et al., 2020).

Peptic ulcer disease is often characterized by a breach in the stomach of the mucosa greater than 3-5 mm or duodenum with a significant depth. It is also an endoscopic disease, which is a medical diagnosis based on symptoms alone, as opposed to dyspepsia. An mismatch between factors that shield the stomach and duodenum mucosa and factors that harm it induces peptic ulcer disease. Similarly, gastric and duodenal ulcer patients are present. It is likely to report epigastric or retrosternal pain, early satiety, nausea, bloating, belching, or postprandial illness. These signs are non-specific and it can be impossible to separate them from functional dyspepsia clinically. In comparison, patients may remain asymptomatic before a condition happens, or an ulcer may be incidentally detected for other causes during endoscopy (Sverdén et al., 2019). Studies have demonstrated a poor association between symptoms and endoscopic performance.

1.3 Gastric Ulcer

Gastric ulcer is a chronic condition that occurs when the mucosal lining of the stomach is destroyed. There is a physiological equilibrium between aggressive factors and mucosal defence. When this equilibrium is broken in place of offensive causes, there is disruption to the gastric mucosa. Any of the violent causes include *H. pylori* non-steroidal anti-inflammatory medications, ethanol and genetic factors. Ethanol has a devastating

impact on gastric mucosa as a result of its use in animal models to cause gastric ulcers (Ofusori et al., 2019).

Gastric ulcer is a condition of the stomach lining that has common symptoms such as vomiting, burning, dull abdominal pain, headache, weight loss, low oral resistance, stenosis, perforation and stomach bleeding (Mashayekhi et al., 2020).

In past years, scientists have sought to explain why infection with *H. pylori* only induces disease in a limited proportion of the affected population and why it may follow a benign path in some people, while in others it is malignant. It seems like the trick to addressing these questions is to assess the quality and severity of the inflammatory response to infection, which in turn affects the evolution of gastric lesions (Fuenmayor et al., 2020).

One of the organs affected by tension at the highest level is the stomach. Any biological and psychosocial situations have been documented to enhance the formation of stomach ulcers. A total of 200,000 patients with ulcer diagnosis are treated on an annual scale; 3 million people are admitted to polyclinics; and the financial burden of treating this disease hits 4 billion dollars. The development of ulcers in the gastrointestinal (GI) system is induced by numerous causes such as *H. pylori* infection, smoking, indomethacin and non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ethanol, stress, free radicals, bile acids, protease, exercise, starvation, cold and immobility (Eraslan et al., 2020).

1.4 Medicinal Plant

The focus has been on natural plants, partially because certain pharmaceutical medicines are highly harmful to the patient or cause adverse effects. Moreover, in terms of cure and disease prevention, plant products are inexpensive and more affordable contributors to improving human health. Over the years, medicinal plants have been considered well-established natural sources for the cure, with or without scientific basis, of different diseases. The World Health Organisation tracks and publishes over 20,000 plant species used for medicinal purposes. Conventional therapy is more culturally appropriate, especially in Eastern countries, and can fulfil therapeutic demands in a way that Western medicine does not (Youssef and El-Mahmoudy, 2019).

Globally, the use of herbal remedies has seen a great upsurge. Many patronize them in developed countries primarily because of cultural acceptability, availability and expense. They are used in developing countries because they are herbal and are hence believed to be better than allopathic drugs. There has been a growing worry about their welfare in recent years though. This has generated a circumstance of ambivalence in the debate of their use. Any medicinal plants are potentially harmful because of their constituents and can cause allergic reactions if they are misused (Mensah et al., 2019).

1.4.1 *Punica granatum*

The potential medicinal properties of indigenous plants have been observed globally and have been used either to cure or to prevent various diseases. A plant of the family Lythraceae, *Punica granatum* (pomegranate), has been reported to have several medicinal properties, including chemopreventive, antioxidant, antifungal, anti-inflammatory, antibacterial, and wound healing. A preventive role against obesity has also been identified. 7 Steroids, triterpenoids, saponins, glycosides, flavonoids, alkaloids, carbohydrate tannins and vitamin C have been found to contain phytochemical screening of *Punica granatum* extract (Nuraddin et al., 2019).

Pomegranate fruits (*Punica granatum*) are as sweet, sour and mild. Both parts of the plant flowers, leaves, buds, stems, seeds and peels are commonly used.

Pomegranate has been used in medicine to treat diabetes, breast cancer, prostate cancer, heart and kidney failure and dental infection (Al-badry, 2019).

Pomegranate is' a treatment for the withdrawal of worms' in traditional Indian medicine. The fruit peel is a strong astringent used to cure diarrhoea. In comparison, anti-fever is the fruit juice. Elements such as calcium, selenium, arsenic, cobalt, chromium, cesium, copper, potassium, magnesium, manganese, molybdenum, sodium, rubidium, scandium, serum, ten strontium and zinc are present in pomegranate juice, which is comparatively more common than others, and minerals such as iron (Hassani and Sepahvand, 2020).

1.4.2 *Hypericum perforatum*

Many plants have long been used in herbal medicine as a consequence of having illness-curing or health-promoting properties. *Hypericum perforatum* L. It belongs to the

Hypericaceae and is generally referred to as St. John's Wort (SJW). It has been used as a treatment in various Eastern countries. Nevertheless, scholars suggest that it was used by ancient Greeks to cure mental illnesses that they felt involved demonic possession. In temperate open disturbed regions, it grows well (Belwal et al., 2019). The *Hypericum* L genus. "St. John's wort" (Hypericaceae) consists of about 500 species of shrubs, trees and herbs, primarily in temperate areas of the Northern Hemisphere, but also in high-altitude tropical and subtropical regions (Meseguer et al., 2013).

For various indications, including skin disorders, St John's wort is used as a folk remedy (minor cuts, burns, skin ulcers, topical viral infections), In the management of inflammation of the bronchi and urogenital tract, and mentally, in the treatment of depressive spells, biliary diseases, pain of the bladder, common cold, diabetes mellitus, dyspepsia, haemorrhoids, neuralgia, migraines, sciatica, and ulcers (WHO Picked Medicinal Plants Monograph. The findings of multiple experiments have shown that H. Perforate has anti-inflammatory, antimicrobial and wound healing effects (Zdunić et al. 2009).

1.5 Omeprazole

Omeprazole is used for the treatment of conditions in which the stomach excretes large quantities of acid. It prevents the activity of a particular enzyme mechanism located within the acid-secreting stomach cells (in the stomach), thus preventing acid production. Omeprazole was being used to control: heartburn (heartburn), acute gastritis, duodenitis (duodenitis), esophagitis attributable to recurrence of gastrointestinal material, peptic ulcer and other secretary diseases. Acidosis, excessive (such as Zollinger Ellison Syndrome). Omeprazole is sometimes used for the removal of Helicobacter pylori-a form of bacteria in the intestine, often briefly and as part of a combined therapy (Sahoo, et al., 2017).

1.5.1 The mechanism of omeprazole’s action

On the cells lining the stomach, where they are used by these cells to manufacture stomach acid, proton pumps were located. Omeprazole functions by blocking the activity

of the proton pumps, thus reducing the production of acid in the stomach and hence the amount of acid in the stomach and duodenum. Omeprazole prevents the return of excess acid to the oesophagus and can be used to alleviate and treat GERD-related symptoms (Safavi et al., 2016). It also makes it easier for peptic ulcers to recover. In order to help remove a form of bacteria called Helicobacter pylori in the stomach of people with peptic ulcers, omeprazole is often given with antibiotics. These bacteria can lead to the development of a peptic ulcer. This medication makes it easier for the ulcer to cure and helps establish a gastrointestinal tract environment in which antibiotics are more successful at killing these germs (Malfertheiner et al., 2011) (Ismail et al., 2012). (Palamides, et al.,2020) (AlRashdi et al., 2012).

1.6 Aim of the study

The purpose of the study to test the effectiveness of *Hypercum perforatum*, *Punica granatum* and omeprazole in gastric ulcer in male albino rats was to decrease the ulcer size by using medicinal plants.

2 LITERATURE REVIEW

2.1 Gastric Ulcer

"The study measured the effectiveness of *Cibotium barometz* (*C. barometzs*) hair in animal hemorrhagic abrasions caused by ethanol. Seven bunches of Sprague Dawley (SD) rats were given 10 percent Tween 20 for the control and ulcer control gather and omeprazole bunches 20 mg/kg and 62.5,125,250 and 500 mg/kg of *C. barometzs*. The barometz hair extraction in the laboratory schools. 10% of Tween 20 was administered orally after 60 minutes in the normal rat control category, while complete ethanol during oral administration in the ulcer control, experimental groups and omeprazole. Macroscopically and histologically, rat stomachs have been examined. In order to measure the activities of endogenous antioxidant enzymes, stomach homogenates were used, Pre-fed rats had minimized ulcer sites, increased pH of gastric, and preserved gastric mucus in comparison with ulcer groups (Al-Wajeeh, et al., 2016, Saeed et al., 2016).

2.2 *Helicobacter pylori*

Bacterial gram-negative spiral infectious agent, gastritis causative agent and peptic ulcer disease, WHO organization identified *H. pylori* as Type I carcinogen, Although *H. Pylori* is one of the most exceedingly bad in Africa within the world, with a comparably low occurrence of gastric cancer. No other *H. pylori* related effects are known. Correlation of bacterial toxicity to these phenotypes with *H. pylori* in African countries (Yu et al., 2018).

Our team has set up a network of research centers in South Africa and Nigeria (NG) for a general image of the epidemiological circumstance (ZA). An add up to of 220 confines from 114 patients were tested and 118 individual isolates have been tested,

phylogenetic origin and susceptibility of the virulence factors *cagA*, *vacA*, *dupA*, to the Amoxicillin, clarithromycin, tetracycline and metronidazole are commonly used as antibiotics. Our team has been reporting that H pylori. In their virulence factor manifestation and phylogenetic profiles, isolate from Nigeria and South Africa vary greatly. *vacA* mosaic is extreme, The outcome was *vacA* chimaeras of m1-m2 and regular subtypes of s1m1 and s1m2 *vacA* in hpAfrica2 strains. Gastric lesions in Nigerian patients were more often diagnosed compared to Patients from South Africa and isolates of *H. pylori* immune to one or more antibiotics are widespread in both nations (Palamides,etal.,2020).

2.2.1 Virulence factor of *H. pylori*

H. pylori are a bacterium that contains gram negative substances that contaminates a number of people worldwide. The etiology of gastritis, peptic ulcer and gastric cancer their causative agent has been found that was *H. pylori*. The pathogens of this bacterium are mainly attributed to the pathogenic genes of island (PAI). Cytototoxin-associated gene A (*cagA*) and vacuolating cytotoxin genes are the most common genes on these islands. Most studies have shown different frequencies of *cagA* and *vacA* in patients with peptic ulcer or gastritis in different countries. These changes in *cagA* and *vacA* abundance can be attributed to the genetic diversity of these bacteria and can affect the geographic diversity of these gene expressions. *H. pylori* infection is usually not associated with any health condition, but it causes gastrointestinal inflammation leading to peptic ulcer and stomach cancer. The function of *H. pylori* in the pathogenesis of gastrointestinal disorders with a focus on *cagA* and *vacA* virulence factors was shown in this study (Nejati et al., 2018, Roesler et al., 2014).

The human gut is persistently colonized by *H. pylori*, with a mixed function in human health. "The *cagA* protein, a major host-interacting factor, translocated to host epithelial cells via the EPIYA tyrosine phosphorylation motif (TPM), the type IV secretion pathway, is identified by host cell kinases coming about in different have cell signaling cascades". The *cagA* TPMs, each with a clearly preserved amino acid sequence surrounding the EPIYA, are Type A, B, C or D. The search in the database showed that B-motif distribution in Western Helicobacter Pylori isolates was non-random (including

EPIYT and EPIYA). The Silico *H. pylori cagA* sequence from Western studies showed that EPIYT B-TPM was much less related to stomach cancer than EPIYAB-TPM. By Using of special phosphorylated antibodies against *cagA* B-TPM (Zhang et al., 2012).

The *cagA* B-TPM EPIYT phosphorylated state was demonstrated in the study. Co-culture of *H. Pylori* and host cells. Phosphoinositol 3-kinase (PI3-kinase) *cagA* interactions were also shown to be host cell-dependent on B-TPM tyrosine-phosphorylation and EPIYT B-TPM recombinant *cags* have greater PI 3 kinase affinity and stronger AKT induction than isogenic *cagA* with EPIYA B-TPM.

The PI3 kinase-linked *cagA* B-TPM motif shows Threonine residues at PY+. Structural modeling of AGS cells during co-cultivation. Helicobacter Pylori strains with CagA EPIYT B-TPM significantly reduced the generation of interleukin-8 and hummingbird phenotypes similar to homologous strains with B-TPM EPIYA. These findings indicate that A/T polymorphisms can control the action of CagA by interfering with the host's carcinogenesis signalling pathways that affect cancer risk. (zhang et al., 2015).

2.2.2 *H. pylori* associated disease

2.2.2.1 Peptic ulcer disease

Acid-induced lesions in the duodenum and stomach are peptic ulcers, by naked and defective mucosa extending to sub-mucosa or propria muscularis. Erosions are considered lesions which do not exceed this depth. In the United States the Prevalence of auto determined peptic ulcer disease was 10% in 1990, and the estimated occurrence is around 500,000 new cases each year. Overall, however, globally, the risk of death and need for hospitalizations related to PUD has decreased. That was most mutual suspicion to the reduction in infections of Helicobacter pylori (H. pylori) due to medication and better grooming. This pattern may also be partly attributed to expanded use of prescription and over-the-counter acid-suppressing medications and greater vigilance of non-steroidal anti-inflammatory drugs (NSAIDs) (Narayanan et al., 2018).

2.2.2.2 Gastritis

Long-term gastritis and gastric ulcers are widely reported around the world and are a global problem for health. In traditional hospital medicine, the modified herbal Xiao-Chai-Hu Tang (XCHT) is widely used to treat gastritis. In order to evaluate the anti *H. pylori* effect of MXCHT, the in-vitro method of agar diffusion and the minimum concentration of fluid dilution (MIC) have been developed. A model of a mouse oedema and a rat paw edoema tested the anti-inflammatory effects. A gastric ulcer technique induced with ethanol was used to confirm the gastric protection function of MXCHT active extracts. HPLC-TOF-MS/MS was used to assess potential active ingredients following oral administration of useful extracts in the ethanol-induced gastric ulcer model. Compared to the control group, the MXCHT and 4 distinct inhibitions of bacteria and MIC extracts greatly decreased, indicating anti *H. pylori* special effects. Dose Strong of MXCHT, extract of water, extract from EtOac, and BuOH Extract have shown substantial anti-infection efficacy in the xylene-induced oedema model, and the carrageen-induced rat paw model test for oedema. MXCHT was also shown to be gastro-protective in all efficient extracts, avoiding gastric lesions in rats that were ethanol induced. Following four prototype components and 4 metabolites have been found for oral administration of EtOAc extract. In addition, in the -BuOH extract was composed of six prototype components and six metabolites. The gastro-defense effects of MXCH T, EtOAc, and -BuOH extract are demonstrated by anti *H. pyloric* and action against inflammation. For chronic gastritis and gastritis prevention and care, this drug can also be an important natural source (Chen et al., 2018, Jia et al., 2009).

2.2.2.3 Gastric malt lymphoma

Lymphoma of gastric mucosa combined with lymphoid tissue (MALT) is an uncommon condition, and most available gastric MALT lymphoma (GML) results are obtained from clinical practice of chosen patients treated in centres of greatness. The research seeks to examine the medical characteristics, treatment and patients' survival with GML in a survey focused on the population conducted in France. This included all new GML cases in 11 French cancer registry regions diagnosed between 2002 and 2010.

Pathology findings have been checked by an expert pathologist and, if applicable, reviewed. Both clinical data is retrospectively obtained and analysed using stata V. 14 tools from medical reports. Outcomes Four hundred and sixteen patients (50 percent male, median age 67 years) with reported GML were reported. Among them, 44 demonstrated an early transition into diffuse large B cell lymphoma and were deemed to have had a high-grade lymphoma that was originally overlooked. 76 per cent of patients were in stage IE/II at presentation, and 24 per cent were in stage III/IV of the disorder. In 57 percent of the patients, Contamination of H. pylori was observed. 76 percent of patients were given enucleation therapy and 39 percent got full re mission (CR). One hundred and ninety patients, including 10 still in CR after enucleation, received at least one other medication. In 70 percent of patients, CR was obtained and the 5-year average survival was 79 percent (95 percent CI [75-83]) (Matysiak et al., 2019, Zeng et al., 2020).

2.2.3 Diagnosis of *Helicobacter pylori*

Gastritis *H. pylori* infection with atrophy is not well known in best biopsy-based testing sites. The target of the task was to evaluate this study and sensitivity in terms of atrophic gastritis in biopsy-based studies. There were 144 patients with dyspepsia that were not examined (164). No-invasive experiments (anti-*H. pylori* IgG) were conducted and tests based on biopsy (i.e. culture, histology of Giemsa, stain and rapid urease test). According to previous recommendations, the *H. pylori* infection gold standard has been created. Due to sensitivity, specificity, a positive predictive rate and a negative predictive rate the degree of gastritis was tested. of gastric antrum and body biopsy-based studies (Genta and Graham 1994).

The findings include the prevalence of *H. pylori* In 164 patients, the infection of *H. pylori* was 63.4 percent. Gastritis was significantly higher in the antrum than in the body (76% vs. 31%; $p<0.001$). Sensitivity to the biopsy test decreased, with the degree of atrophy gastritis increasing regardless of biopsy position (the sensitivity of histology Giemsa stain increased to 100 percent for normal, moderate, modest and extreme gastritis with atrophy 100 percent, 88 percent, and 66 percent, respectively, and 100 percent, 97 percent, 91 percent, and 66 percent, respectively, for rapid urease test). A further body

biopsy resulted in an increased sensitivity of 16.67 percent in serious to grave gastritis of the antrum or body with atrophy in relation to a single antrum biopsy (Lan et al., 2012).

2.2.3.1 Comparison between all four type of invasive method

The object of the research was to test four *H. pylori* Diagnostic approaches for infection in dyspeptic patients. In this cross-sectional descriptive analysis, 165 specimens of antrum biopsy were obtained from dyspeptic patients referred to and collected in 2018 by the endoscopy unit of Shariati Hospital, Isfahan, Iran. *H. pylori* were tested for each patient, including histology, culture, rapid urease test (RUT) and fluorescence in situ hybridization (FISH) (Kalali et al., 2015).

The study's gold standard was one of the two measures, RUT, or histology, for positive confirmation. The occurrence of *H. pylori* contamination was 55.2 percent according to the predefined criterion. Of the four diagnostic approaches, FISH and RUT, respectively, were the most susceptible (95.7 and 92.3 percent). Given the high histological analysis precision (100 percent), its NPV was lower than that of the other methods (88 percent). The kappa coefficient of agreement was perfect ($P < 0.001$) between the gold norm and the techniques evaluated. In addition to the diagnosis of H, FISH and histology are prescribed. Infection of pylori, which can optimally control the complications (Vazirzadeh et al., 2020).

2.2.3.2 Non-invasive test

The diagnostic precision, independently or in combination of the urea breath assay, serology and stuff antigen test, for symptomatic and asymptomatic diagnosis of the *H. pylori* infection patients should be compared in order to begin *H. pylori* eradication treatment. The outcome revealed that urea breath tests had high diagnostic accuracy in persons devoid of a olden times of gastrectomy and individuals who has not in recent times consumed antibiotics or proton pump inhibitors, whereas serology and stool antigen tests were less successful for Helicobacter pylori disease determination. This is dependent on an indirect test analysis (with possible prejudice due to confusion) as data from direct comparisons has been minimal or inaccessible. The parameters usage for these

experiments were widely complex and we do not do it defines particular thresholds that could be helpful in clinical practise (Best et al., 2018).

2.2.4 Treatment of *H. pylori*

From June 2018 to May 2019, a regional medical institution has reported patients who had been contaminated with Helicobacter pylori infections with standard 14-d quadruple treatment and undergoing a curative test. Recorded population and clinical data. Rates of eradication were assessed and the regimens were matched between subgroups. The predictors of eradication failure were listed in a multivariate regression. In 1999 (76.6 per cent), a total of 2610 patients were eradicated successfully. Quadruple regimes with apoxicillin reported a higher eradication rate than others (83.0% vs 69.0%, $P < 0.001$).

Various amoxicillin-plus clarithromycin therapies achieved the quickest eradication pace (83.5 percent). Higher eradication rates than rescue therapy are found in primary therapy (78.3 percent vs 67.5%, P<0.001). The amount of amoxicillin and furazolidone consumption during the rescue therapy was the largest deletion (80.8 percent). Esomeprazole-based regimens displayed a higher eradicative performance than other proton pump inhibitors (74.9%, P = 0.001%, 81.8% vs 74.9%). The high risk for eradication error was related to older age, prior medication, or omeprazole or pantoprazole use through multivariate regression analysis (Yan et al., 2020, Suzuki et al., 2020).

2.2.4.1 Comparison between high dose dual therapy (HDDT) and versus bismuth quadruple therapy (BQT)

"The goal of this study was to systematically evaluate and examine *H. pylori* infection with high doses of double medication (HDDT) for quadruple bismuth medication (BQT)". In Chinese up through to March 2018, the solution is to compare HDDT to BQT from Pub Med, EMBASE, Cochrane Archive, CNKI, and Wanfang databases. The efficacy and side effects of these 2 H pylori-infection therapies were compared using Review Manager 5.3 for statistical purposes. The dichotomical data was pooled to calculate relative risk (RR) with a 95% confidence interval (CIs) (Zhu et al., 2020).

The evaluation has been performed on four randomized clinical trials (RCTs) with 829 H-pylori patients. Overall, the meta-analysis found that the rates of productivity eradication for both HDDT and BQT were similar, respectively, to 85.5%, vs 87.2%, RR 1.01 (95% of CI 0.96-1.06), P =63% and of eradication for protocol (PP) RR 1.00 (95% of CI 0.96-1.04), P =97% and 97.8% compared to 95.0%; RR 1.01 (95% of RR 1.1). HDDT and BQT can have a similar rate of eradication and HDDT usually has less side effects (Yang et al., 2019).

2.3 Plants as a source of medicinal compounds

It is possible to identify medicinal plants as plants with healing properties or a positive pharmacological effect on the human and animal body (Namdeo, 2018). Naturally, medicinal plants synthesize and accumulate certain secondary metabolites that are responsible for certain behaviors or act as a significant therapeutic effect on the human body. Herbal treatments include extremely active pharmacological factor metabolite molecules that are widely utilized for the care, risk mitigation and prevention of several diseases. In addition, certain medicinal plants and medications are stable at one dosage, but dangerous at a different dose (Briskin, 2000, Saad et al., 2006).

2.3.1 An overview of the genus *Hypericum spp.*

The species is found in wild wild nature in Spain, France, Greece, Cyprus, Turkey, north Syria, Lebanon, Palestine, Jordan, Egypt, Iraq and northwest Iran (Robson, 2002).

Natural product science has evolved in recent times in a clear and accurate way to classify substances for the treatment of infections such as depression, heart failure, inflammation, and even the discovery of a natural product with active antiviral and anticancer activities. St. John's wort or *Hypericum perforatum* is used as an ancient herbal cure for various diseases (Kirakosyan et al., 2008).

The *Hypericum* genus belongs to the family Hypericaceae, which contains more than 500 species (Meseguer et al., 2013).

Perennial, medicinal or scrubby plants are hypericum. In Hypericum, since it is the source of multiple molecules with varying biological activity, there is a growing significance (Ayan et al., 2004).

The species of hypericum range from small trees and shrubs to grasses. Although this genus is wide-ranging, it is not especially dry, warm or cold and scarcely found in water in environments. Thus, in the desert, polar area and tropical lowlands (Ernst, E) (Ed. 2003).

2.3.1.1 Hypericum perforatum

Another study was conducted to test the protective against gastricity activity of *H. perforatum* leave extricate in rat gastric ulcer-induced ethanol compared to esomeprazole (the sedate of choice for stomach ulcers). The process of action was carried out according to the Auto Dock Vina system. The inflammatory reaction was mediated by ethanol ingestion, as seen by an increase in gastric pro inflammatory TNF-alpha with a decrease in IL-1β. In the other hand, the presence of alkaloids, flavonoids, tannins, phenols, steroids and saponins was revealed by HP's phytochemical screening. In comparison, significant mucosal loss damage of the ulcer management rat population is found in the high dose of the HP group, which indicates moderate injury the stomach mucosa that is equivalent to the community esomeprazole. Data from In silico appeared that Amentoflavone and Quercitrin have the most noteworthy partiality and exceptionally strong associations with the active site of НЯ/Kş ATPase al. This research found that HP is almost as efficient as esomeprazole in preventing the plant extract from gastric ulcer caused by ethanol and is more closely related to gastric proton pumps than esomeprazole (Sofi et al., 2020, Ali et al., 2014).

For centuries, restorative plants have been broadly utilized in wound mending of burn wounds. histological parameters, reepithelisation, fabric of granulation development, aggravation, and angiogenesis were examined biopsies on the end of the scar the study. There was a statistically important discrepancy between all the control community histological parameters and that of the other bunches ($p < 0.05$). Here was no measurably meaningful change in terms of re-epithelization and inflammation between groups B and C ($P = 0.351$, $P = 0.067$). A substantial differential was found in the curcumin community relative to the HP group for two factors of fibrosis and angiogenesis ($p<0,05$). Both curcumin and *Hypericum perforatum* oil are involved in the healing of wounds. Our

studies have shown a higher health-giving efficiency in treated rats with curcumin (Seyhan, N. 2020, Kıyan et al., 2015).

Derivation of the name *Hypericum perforatum* The root of the family's Greek name, Hypericum, is unknown. One version is that it comes from the "hyper eikon" ("exceeding any imagination") and refers to the great healing ability of the herb. It is more likely, however, to be attributed to the Titan Hyperion ("the higher"), whose union with Theia created the sun god Helios. Helios (also often referred to as Hyperion) wears a crown of rays and is called the 'illustrious.' The name of the genus "perforatum" refers to the flowers and leaves that appear as though they were perforated with needles. (Mullaicharam and Halligudi, 2019).

2.3.1.2 Morphology of *Hypericum perforatum*

Erect, annual grass 30-90 cm high replicated on the base of a stem by seeds or rhizomes. Tubes of black glands, a hairless ring at the lower nodes are very slanting, 2-sided, or narrow along the ridges. Simple, pinnate net, opposite, stalkless, typically teethless, hairless with straight points, apex circled. Garish, yellow flowers in fairly smooth clusters at the top of the vine. Sepals 5, lance, grey, 5 mm in length, 1 mm in width. Petals 5, black glandular bordering gold, 14-15 mm long, 8 mm width. Seeds of l.l x 0.50 x 0.4 mm, black, reticular, with small sharp ends, several stamens; one 3 part and broader divergence pistil; fruit for each capsule (Robson, 2002).

Figure 2.1 (a) Leaf of *Hypericum perforatum* (b) Flower of *Hypericum perforatum*

Figure 2.2 Whole plant of *Hypericum perforatum*

2.3.1.3 Classification of *Hypericum perforatum* (Velingkar et al., 2017)

- Kingdom: Plantae
- Division: Tracheophyta
- Subdivision: Spermatophytina
- Class: Magnoliopsida

- Order: Malpighiales
- Family: Hypericaceae
- Genus: Hypericum
- Species: Hypericum perforatum

2.3.1.4 Chemical compound of *Hypericum perforatum*

The high-performance liquid chromatography analysis (HPLC) of polar inputs was tested in air-dried plant samples, and the GC-MS tests for EO elements were carried out by means of solid-phase micro extraction. Liable on the elevation in both uplands, all of the polar phytochemicals examined were identified at different levels and the accumulation frequency of the positive of each molecule stable and meaningful reaction to the gradient altitudinal (Guo et al., 2018).

The main EO components based on altitude have also been observed for their substantial consistency or quantity: 2,2,6-trimethyl-cyclohexanone, caryophylene oxide, tricycloteptane, hexanoic acid, tetradecanol, 2-methyl-dodecane, tetradecane, α-amorphene, eicosene, β-cedrene, piperitone, spathulenol, β-caryophyllene and linalool. The present results suggest that the plant habitat altitude ultimately affects *H. perforatum* secondary metabolism and show new chemo types in terms of the EO composition of investigated populations. Such results could help to identify the specific strains with the desirable composition of the chemical and fresh perspectives for study in this precious medicinal plant (Seyis, et al., 2020).

2.3.2 Used in traditional medicine

The ancient aerial portion of St. John's wort is medically used. It is used to decrease fever, remove toxins, reduce bleeding and alleviate muscle pain. In traditional Chinese medicine, at least 30 distinct genus members are included (Holstege, et al., 2005).

2.3.3 Biological features of *Hypericum perforatum*

2.3.3.1 Antimicrobial activity

This research explores ethanol excerpt (HP-EtOHantimicrobial) activity. *Hypericum perforatum* and its subsectional excerpts, including N-hexane (HP-hexane), chloroform (HP-CHCl2) extracts, and *Streptococcus mutans* (H.Sobrinus, Lactobacillus Plantarum, S.Sobrinus) extract and *Enterococcus faecalis*, and N-butanol (HP-n-BuoH). Floring aerial sections are needed for an estimate of antimicrobial activity. *H. perforate* was extracted with EtOH, and then this extract was broken down to five separate polarity sub-extracts. HP-EtOH and its sub-extracts for *Streptococcus mutants, S. sobrinus*, L, antimicrobial activities. The colored micro-well dilution at 64–0.5 mg/ml concentrations and the adjusted micro-titer plate range of 100-0.78125 mg/ml was used to measure plantar and *E.faecalis* (Peeva et al., 2013).

Based on the findings of this analysis, The HP-H2O sub-extract shows excellent antibacterial activity (MIC 8 mg/mL) against *S. sobrinus and L. plantarum*, and *S. mutant* and *E. faecalis* are present at concentrations of 32 and 16 mg/mL, respectively. "Another sub-extract also showing antimicrobial activity against *S. sobrinus* at a concentration of 16 mg/mL. HP-EtOAc and HP-n-BuOH showed antibacterial activity against *L. plantarum*, while HPEtOAc and HP-H2O were also successful against *E. faecalis* at the same concentration (16 mg/mL)". Research has suggested that *Hypericum perforatum* can be utilized as a normal antibacterial operator in verbal care items (Süntar, et al., 2016).

"Three clinical pathogens were measured for the anti-bacterial effect of *Hypericum perforatum* extract. *C. albicans, E. coli* and *S. aureus*. *Hypericum perforatum* extract's maximum antibacterial activity on *S. strains* of *aureus*. As a result of anti-biofilm analysis, *Hypericum perforatum* was also inhibited by 56.85% by biofilm formation of S. aureus. The 92,85 percent reduction in S. aureus biofilm was achieved by the combination of polyurethane and the *Hypericum Perforatum* Extract (PHPE) compared to the control. The S. aureus decrease has been shown by the SEM study (Scanning Electron Microscopy) after *Hypericum perforatum* integration. The findings indicate that the substance in polyurethane with the extract of *Hypericum perforatum* inhibits *S. aureus* biofilm formation" (Nazlı, et al., 2019).

2.3.3.2 Anti-inflammatory process

Inflammation is a biochemical reaction of the immune system that can be caused by a broad range of stimuli, including bacteria, infected cells and toxic substances. Inflammation is characterized by redness, swelling, heat, pain and tissue loss because of local cell, immune and vascular reactions to infections or injuries (Chen et al., 2018).

Hypericum perforatum L oil extracts was prepared under the medical recipe of traditional medicine in three different types. The composition variability and biological role of the oil extract have been analyzed. The test was utilized for anti-inflammatory testing of carrageenan-induced rat paw oedema and the test was used to assess protective gastrointestinal function by indomethacin-mediated gastric rat mucus destruction (Tanideh et al., 2020).

There was anti-inflammatory and gastro-protective activity in all the oil extracts examined. The maximum anti-inflammatory effect (95,24 ± 11,66 per cent) and gastro-protective effects (gazing injury score of 0,21 ± 0,12) were among these extracts prepared with 96 per cent maceration ethanol, accompanied by withdrawal of flower light oil with warming in a water bathtub (extract 2) at a dose of 1,25 mL/kg. The largest concentrations of quercetin and I3, II8 biapigenin have the same oil extract (129 ± 9 μg/mL and 52 ± 4 μg/mL, respectively). Anti-infectious activity similar to that of indometacin was demonstrated by Quercetin and I3,II8 biapigenin as well as strong gastro-protective activity. The results reinforce the use of Oleum Hyperici as an anti-inflammatory and gastro-protective agent that has traditionally relied primarily on ethnopharmacological claims (Zdunić et al., 2009).

2.3.3.3 Anti-viral

The study has analyzed *H. perforatum* antiviral activity on infectious bronchitis viruses for the first time. The study's results confirmed *H. perforatum* was a component of antivirus and suggests that HPE and IBV titer messenger titer therapy with ribonucleic acid (mRNA) greatly deteriorated, with a drop in positive Green IBV immunofluorescence signal in the cells in the chicken embryo kidney (CEK). HPE-treated doses of 480 – 120 mg/kg over 5 days reduced IBV damage and kidney damage, in addition to minimising

IBV mRNA expression in the trachea and renals in vivo. Our study reveals that in vitro and in vivo HPE has considerable anti-IVV effects with a substantial decline in IL-6, alpha-tumor (TNF-α) and kappa beta nuclear factor (NF-α) expression respectively (Chen et al., 2019, Xiuying et al., 2012).

2.3.3.4 Antioxidant activity

The purpose of this paper is to analyse the influence on the polyphenol content of plants of successive milling and sewing processes. The total phenolic content and antioxidant activity were evaluated, as well as some bioactive compounds detected and quantified with LC-ESI/MS. For the 100-180μm fractions, the maximum antioxidant activity was reached, IC50 was 0.43 and 0.51 mg/mL for H. A and perforate. Millefolium. Millefolium. These findings demonstrate that the fine smear and sevens contribute to a differential bioactive compound distribution by particle size (Becker et al., 2016).

Ethanolic extract of the Tlc-DPPH and DPPH assays displayed a spectrum of 5.9 percent, respectively, and an antioxidant activity of more than 70 percent (0.1 mg/mL) under Sonicity. Ethanolic extracts from the *Hypericum perforatum* (Hypericaceae) were obtained. In the HPLC review, the high concentration of *Hypericum perforate* extract (2.76 mM). Drive through an ultrasonic ethanol extraction from medicinal plants to illustrate a basic laboratory technique. This feasible didactic method has been demonstrated to restore antioxidant function rapidly and effectively (Nicolai et al, 2016).

2.3.3.5 Antidepressant activity

Efforts are identified and explored to classify hyperforin as an antidepressant part of therapeutically used alcoholic extracts. This extracts have been extremely rich in hyperforin (38.8%) and are free from hypericin and many other alcoholic extract components. These attempts demonstrated its antidepressant efficacy in the therapeutic desperation testing, leading to the working hypothesis that the antidepressants in alcoholic hyperic extracts contain hyperforin and serotoninergic processes. The findings also show that hyperforine is the only antidepressant ingredient, but not the only part of alcohol (Chatterjee et al., 1998).

2.3.4 Other activities of *H.perforatum* on some organs

2.3.4.1 Effect on the liver organ

Apparent feature of *Hypericum perforatum* are anti-inflammatory and antioxidant. They are very important properties to protective hepatic damage. The study of, Aydin et al (2014) in hepatic ischemia-reperfusion model, it was illustrated that extraction of *Hypericum*, have protective affect to decrease lipid peroxidation and inflammatory cytokine level. Furthermore, mainly tissue damage caused by increased production free radical or ROS. Also, can be detected by malondialdehyde measurement. Hepatic ischemia-reperfusion injury is usually related to high morbidity and mortality. In another analysis, *Hypericum* is capable of reducing malondialdehyde (MAD) protective damage and of increasing CAT and GSH enzyme activity (Bayramoglu et al., 2014). Applied at various dose amounts 25, 50 and 100 mg/kg of *Hypericum perforatum* with antioxidant activity, *Hypericum perforatum* demonstrates a substantial decrease in ALT, ALP and LDH levels as a defensive effect against oxidative stress (Yildiz et al., 2018).

2.3.4.2 Effect on the kidney organ

The excreting and regulatory functions of the kidney in order to ensure that the blood fluid is consistent and is an active part of endocrine and metabolic activity in the environment of the cells of the body. It generates renin which plays an important role in intrinsic pressure controls and erythropoyetins, and in the general pressures to improve erythropoesis. The mammalian kidney's primary purpose is to process waste material and surplus blood fluid through and release of certain hormones and successful vitamin D3 synthesis. Regulation of water level and electrolytes during the day is one of the primary functions of the renal as it seeks to keep the extracellular fluid in equilibrium (Santoro et al., 2015, Johnston and Pollock, 2018)

As a common medicine, Hypericum has been used since it contains a variety of bioactive compounds such as flavonol, such as rutin, hyperoside, isoquercitrin, quercitrin, naphthodianthrones, such as hypericin and pseudohypericin. In addition, much of the hypericum perforatum is considered a potent antioxidant. Hyperforin and hypericin are the main constituents of hypericum perforatum that can inhibit activated B cells with the

nuclear factor kappa-light-chain-enhancer (NF-kappa B activation) (Kraus et al., 2010, Laggner et al., 2007).

Neshat et al. (2011) study showed that serum nitrogen urea and creatinine had increased in the one-sided ureteral hindrance show of the rat, but treated with Hypericum perforatum for renal obstruction had similar effects with vitamin E, which could protect renal damage by lowering serum urea and creatinine levels. In addition, Hypericum perforatum was found to be renoprotective by attenuating oxidative stress.

2.3.4.3 Effect on lipid profile

Orthodox Chinese drugs are plentiful causes of organically lively compounds that can be used to deter humanoid disease. Currently, a growing quantity of trials obligate concentrated on herbal extract or natural product, and several of the research concerned identified potent herbal drugs for non-alcoholic fatty liver disease (NAFLD) (Yao et al., 2016).

Hypericum has been used to reduce the lipid profile as another herbal remedy. In the research of Moghaddam et al. (2016), ethanol extraction of *H.perforatum* by maceration process prepared and 300 mg/kg of crude extract subcutaneously administered for two-week hyperlipidemia rats allows cholesterol and LDL levels to be decreased. The popular Chinese medicinal herbs tradition cure, Qushi Huayu Decoction (QSHYD), used in its component japonicum species, finds that (QSHYD) is capable of opposite high free fatty acid levels and total triglycerides (TGs), and hepatic steatosis and inflammation can be increased (Feng et al., 2013).

2.3.5 The side effect of a common component of *Hypericum*

Phototoxic are light triggered secondary compounds that exist in various families such as Hypericaceae, Astereaceae and Brassicasceae (Aucoin et al., 1995). Photosensitization occurs as vegetables are eaten by livestock. The compound in the green plant is at its maximum concentration and is easily ingested from the gastrointestinal tract to circulate in the blood. These compounds react with UV light to create radiant energy in non-pigmented skin that oxidises essential amino acids in skin cells (Knight and Walter, 2003).

Hypericin is the typical photodynamic pigment used in many Hypericum plants. It is a red fluorescent present in black dots, primarily on the surface of the flower leaves, but also on the stem. It is known as the pigment for photosensing, the majority of species are affected by sunlight and feeding. Some animals, including goats, cattle, horses and livestock, are affected. Extreme pruritis (head, ear, face, limbs) and ulcerated, blind and diarrheal dermatitis (Quinn et al., 2014).

2.3.6 An Overview of *Punica granatum* (Pumegranate)

Punica granatum is a natural deciduous shrub in Iran. Today, its medicinal properties have not only been used as vegetables, but have been the subject of researchers in many countries. Granates have medicinal activity for example antimicrobial and anti-inflammatory. Granate seed oil prevents cancers of the skin and breast. Grenate seed oil is rich in compounds of phytoestrogen and phenolic compounds have a strong antioxidant effect (Shaygannia et al., 2016).

2.3.6.1 *Punica granatum* (Pomegranate)

The goal of the research was to explore impact of the watery extricate of pomegranate peel on the treatment of gastric ulceration that was experimentally induced in laboratory rats. Forty adult female rats have been classified randomly into five groups, with eight in each group. At concentrations (100, 50, 25 mg/kg body weight), aqueous extract of pomegranate peels. The findings revealed that indomethacin induced gastric ulcer and certain histopathological modifications involved muscularis layer inflammation, fibrosis, inflammatory cell invasion with epithelium layer transition and blood vessel congestion, whereas the use of pomegranate peel aqueous extract at concentrations (25, 50 and 100 mg/kg body weight) resulted in natural mucosa reconstitution of the stomach (Al-badry, F. A. M.).

Derivation of the name *Punica granatum*, the pagan root of the name pomegranate. “The fruit is known in classical Latin as malum punicum or malum granatum (also melogranatum). Malum means fruit in these names, granatum is granite, meaning (multi)grain (alluding to the many seed grains). In Asia Minor, but in relation to Carthage, the Phoenician colony in North Africa, the adjetive punicus rightly matches Phoenicia

(also the only source of Silphion in Rome)", was more commonly used in Latin; pomegranate was suspected by the Romans of being of African descent. The botanic genus Punica is the feminine form of the adjective, as it is suitable for a herb with a fruit (Chandra et al., 2010).

2.3.6.2 Morphology Classification of *Punica granatum*

Estherlydia (2019) presented that the classification of *Punica granatum* as following:

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Rosidae
- Order: Myrtales
- Family: Punicaceae
- Genus: Punica
- Species: granatum

(a) (b)

Figure 2.3 (a) Fruit of Punica granatum, (b) Leaf of Punica granatum.

Figure 2.4 (a) Flower of *Punica granatum*, (b) Whole plant of *Punica granatum*

2.3.6.3 Chemical bioactive compound of *Punica granatum*

The *Punica granatum* L. plant It has many biochemical functions and a tremendous ability to treat and prevent chronic diseases. To this end, the purpose of this work is to enhance the phytomedicine benefit of the fruit peel of this plant by quantifying and identifying its bioactive compounds and assessing its antioxidant and anti-crystallization activities against calcium oxalate. The findings observed indicate the similar value of the two plant extracts in the removal of free radicals, the inhibition values of calcium oxalate crystallization are 98.11 ± 0.17 and 98.22 ± 0.71 percent against nucleation and 88.98 ± 0.98 and 88.78 ± 2.48 percent against aggregation, respectively, for E.PG and I.PG. These findings show the abundance of the plant in bioactive compounds, providing antioxidant and anti-corrosive properties; thus, it it could be used within the action and /or avoidance of stone forming (Kachkoul et al., 2020).

Bioactivity-based classification of aqueous extracts of pomegranate peel was carried out using various solvents of increasing polarity. Product fraction and residual fraction dissolved in ethyl acetate, butyl alcohol. The main chemical composition of the active component was investigated by HPLC/ESI MS. This study explores how pomegranate peels protect against ricinus oil caused by diarrhoea. The findings demonstrate that the

fraction of ethyl acetate is an active proportion of punica granatum peels that have the anti-diarrheal effect of aqueous extracts with punicalagin, corilagin and ellagic acid (Zhao et al., 2018).

2.3.7 Biological features of *Punica granatum*

2.3.7.1 Antimicrobial activity

According to some studies different sections of the grenade fruit, especially peeling, are recognized by the high level of tannin, particularly punicalagin, contained in grenade extracts as the principal trigger for antimicrobial activity. As the chemical composition of the pomegranate peel can differ with the type of cultivar (sweet, sour-sweet and sour), The findings of this study indicate that the quality of ellagic acid has an important effect on the antimicrobial activity of the extracted pomegranate. A good source of anti-fungal and antibacterial chemicals, PTO8 cultivar pomegranate is a substitute for synthetic antimicrobials (Rosas et al., 2017).

To verify the anti-microbial effect on dental cavities known by micro-organisms to be the most effective etiological agent of pomegranate hydroalcoholic extracts (Punic Granate) The production and management of S effectively hinder peeling extracts. ATCC 25175 R. mutans. Dentocarios, MIC and MBC values medicinal extract 10 µg/µl and 15 µg/µl. The extract of pomegranate juice was also extremely inhibitory against S. ATCC Mutations of MIC 25 µg or µl and MBC 40 µg/µl and ATCC 25175 mutanes. (Odabaş-Serin *et al*., 2020). In vitro microbiological experiments show that pomagram juice and peel hydro alcoholic extracts may compare the main cariogenic bacteria involved in tooth decay. Our results show that pomegranate polyphenolic compounds can provide a good help for the treatment and prevention of dental caries (Ferrazzano et al., 2017, Pagliarulo et al., 2016).

2.3.7.2 Anti-inflammatory disease

The effects of a certain phephaeum (poly) phenolic profile to cure pain and gastric damage caused by anti-inflammatory medicine were used for centuries in the treatment of inflammatory conditions (*Punica granatum* L.). This analysis examined the systemic

effects in mice of formalin-induced nociceptive activity on different doses of HPLC-type granate extract. In the presence of pomegranate, which was also covered against ethanol-caused gastric lesions, indomethacin-induced gastric damage did not occur. The new results support the benefits and the anti-inflammatory effects of polygranate phenolics for treating pain (González et al., 2015).

2.3.7.3 Anti-viral

The Simplex Herpes Virus – 2 (HSV-2) induces lifelong, persistent host infection and, sporadic, HIV transmission among healthy people with high neonatal morbidity. Fruits of *P. granatum* are rich in large bioactive and possible antimicrobial compounds. Therefore, the potency of lyophilized extracts and the bioactive extracted compounds from fruit pelts of *P. granatum* have been tested against HSV-2. This resulted in substantial inhibition of ethanol peel at (62.5 μg/ml). The ethanol extract of the fruit peel was subjected by bioactivity-guided fractionation to the isolation of bioactive compounds. Punicalagin demonstrated 100% anti-HSV-2 activities of 31,25 μg/ml among isolated bioactive compounds with supporting proof of the desirable ADMET silicon properties and good interactivities with selected HSV-2 protein targets through docking (Arunkumar and Rajarajan 2018, Houston et al., 2017).

2.3.7.4 Antioxidant activity

In the Middle East in particular in Iran, pomegranate (*Punica granatum* L.) is commonly planted. It has been used in herbal Iranian medicine for hundreds of years. Granate pelts, seeds and juices produce large quantities of phenolic and antioxidant compounds. The pomegranate peel was tested much higher in overall phenolics, flavonoid and flavonol than seeds and juice. There has been a strong positive association between antioxidant behaviour and total phenolics. Based on the results obtained, the high antioxidant potential of pomegranate, in particular, was seen as a natural preservative of food (Derakhshan et al., 2018).

2.3.7.5 Liver and kidney organs

In many countries, pomegranate is commonly used as medicines. The point of this inquire about was to examine pomegranate antioxidant properties in rats' hepatic and renal tissues. Eighteen male adult rats were classified randomly into three categories, six of which were rats. Granate doesn't have an effect on the role of the liver and kidney. The present evidence shows that lipid peroxidation and nitric oxida in liver and kidney tissue homogeneous have been decreased by PJ and MEPP. There was a large improvement in the dismutase of superoxides and catalase activities of the pomegranate obtained by rats. These results indicate that pomegranate has a strong antioxidant impact (Moneim et al., 2011).

2.3.7.6 Side effect activity of *Punica granatum*

In this research, the safety and tolerability of BALB/c mice were investigated. The classification of a total of 25 BALB/c females was spontaneous. There were five animals in each study group. A single intradermal injection (224 mg/kg) in a single dosage was performed for 22 days, with rehashed measurements like 0,5, 1,9 and 7,5 mg/kg of PPE body weight gavesto BALB/c mice. Intradermal injection has also been conducted for skin allergy testing. Blood was obtained to test the liver toxicity markers of glucose, cholesterol, alanine aminotransferase (ALT) and AST. The tongue, trachea and larynx tissues were also examined on 22 days after management for macroscopic and histopathological purposes. The toxicity ability of EPI research has demonstrated no harmful effects, clinical symptoms, histopathologic effect on tongue, laryngo and trachea epithelial cells, behavioral changes and opposing special effects, or death of BALB/c mice. Repeated administration has not altered or induced oral mucosal inflammation at the local level. In the latter category, skin allergy tests were negative. The latest research indicates that EPI is not harmful and is recommended for use in future disease applications (Jahromi et al., 2015, Vidal et al., 2003).

2.4 Omeprazole

For 40 horses, one of two grades was allocated randomly, with Rank 2 injuries of squamous and/or mucosa glandular. A repetitive gastroscopy test was performed with either Aloe Vera (17.6 mg/kg bwt) (4 mg/kg bwt) in the disease-resolution assessment of a horse (Abood et al., 2020). The performance study is based on a trial of 39 horses. 38 horses have enrolled Equine squamous stomach illness (ESGD); the recovery rates and remedies for these steeds were 56% and 17% for aloe vera and 85% and 75% for omeprazole. **"**Recovery in horses with long stomach draining was less likely.
Equine Glandular Gastric Disease (EGGD) was less prevalent than ESGD (n=14) and the number of glandular stomach diseases was too poor for meaningful statistical analysis". The hypothesis has not been supported that omeprazole does not be less than aloe vera. Aloe vera was less than omeprazole treatment (Bush et al., 2018, Birkmann et al., 2014).

The function of cancer prevention agents and anti-inflammatory behavior's was seen (Xie et al., 2020), according to which this research investigated the synergistically favorable effects of the EMO and PA on GU induced in ethanol in rats. Furthermore, anti-apoptosis, anti-oxidant and anti-inflammatory impacts and protein signalling control have been established for H2O2-inducted cells of the stomach epithelial (GES-1) and LPS-induced RAW 264.7. Consequences established that PA alone or together with OME had major welfares by plummeting sore zones, moderating oxidizing substance stresses, and provocative components, and that dose based healing efficiency alone was shown to be partially better than that of high-dose OME alone.

3 Methodology and Research Design

3.1 Experimental location

This works was performed between August 2020 and October 2020 in the animal house, advanced Microbiology laboratory of the Department of Biology and organic laboratory of the Department of Chemistry, College of Education in Salahaddin University-Erbil, with ethical approval number (140).

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3.2 Equipment and Apparatus and their Manufacturer

No	Name of Equipment	Manufacture
1	Electrical Homogenizer	Germany
2	Blender	Chinese
3	Sensitive balance	Turkey
4	Freezer/refrigerator	Turkey
5	Light Microscope	Germany
6	Oven	U.S.A
7	Micropipette	Ireland
8	pH Meter	Holland
9	Calipers	Germany

10	Formalin	Turkey
11	Ethanol	Chinese
12	Gavage feeding	Chinese
13	Specimen Container	Turkey
14	Slide	Chinese
15	Cover slip	Chinese
16	Incubator	Germany
17	Hood Cabinet	Germany
18	Petri Dish	Chinese
19	Beaker	USA
20	Yellow Tips	Jordan
21	Wood sticks	Chinese
22	Aanaerobic Jar	England
23	Tube racks	Chinese
24	Test tube	England
25	Gram Stain solution	Iraq
26	Blood Agar Base	U.K
27	Normal Saline	Germany
28	Human Blood	In blood bank in Hospital
29	Gas Generation (Microaerophilic) Kit	Japan

30	H. pylori Ag rapid test kit	Chinese
31	Brain – Heart Infusion Broth	U.K
32	Columbia Blood Agar	U.K
33	Glycerol	U.S.A
34	Autoclave	U.S.A
35	Vortex Mixer	France

3.3 Animals and Housing

Ethical guidelines were followed for handling and performing experiments on animals healthy adult male albino rats (Rattus norvegicus) were obtained from the animal house center (College of Education, Salahaddin University-Erbil). They kept in spacious polypropylene cages (50cm x 30cm x 10cm) bedded with wood sawdust in the animal house center, standard condition to the animal room were applied by maintained in a control environment of light (12h light/12h dark cycle) by using electronic programmed timer switches and temperature (25 ±3 ºC) and good ventilated place (Dongre et al., 2008).

The rats feed on standard pellet that was formulated by using a computer program depending on the top feed (Lab Company) in Erbil city. Rodent pellet as the following: wheat 66.6%, soya 25.6%, oil sunflower 4.4%, limestone 1.5%, salt 0.63%, methionine 0,158%, choline chloride 0.062% and trace elements 0.05%, for the preparation of the rodent eat, constituent were mixed with water to make dough, pellet was manufactured and gave to the rats.

3.4 Extraction of the Aerial Part of *H. perforatum*

Fresh aerial plants (Stem, Leaf, flower, fruit and seed) of *Hypericum perforatum* collected from Khabat (district of Erbil city located in the west part of the Iraqi Kurdistan Region). Mainly the aerial part of the plant that has a high proportion of buds and flowers

were used and collected in August 2020. The plant was air-dried indoors to protect it from direct sunlight.

A modified method for selection the doses described by (Chen et al., 2018) and a modified method by (Odabaş-Serin et al., 2020) was used for the preparation of the aqueous extract of the fine powdered plant. The dried milled aerial parts at the rate of high dose (25g/250ml), Low dose (25g/500ml) mixed with distilled water (85Cº), kept for (24h) and filtered with filter paper whatman No.1. Finally, the filtered extract was kept inside sterile cup in refrigerator until used.

3.5 Extraction of Peel Part of *Punica granatum*

Fresh Pomegranate fruit collected from market in Erbil city. Mainly the yellow peel are separated of the pomegranate fruit were collected in August 2020 are used in the study. The samples were air-dried indoors to protect it from direct sunlight. The method of selection the doses described by (Chen et al., 2018) and (Odabaş-Serin et al., 2020) was used for the aqueous extract preparation of the fine powdered samples. Hot distilled water (85ºC) added and left overnight to prepare high dose (25g/250ml), low dose (25g/500ml), then the extract filtered by filter paper whatman No.1. Finally, the filtered extract, were stored inside sterile cup at refrigerator until used.

3.5.1 Omeprazole

Omeprazole is the class of medicines that have been used to treat disorders including peptic ulcers as proton pump inhibitors. Omeprazole inhibits the acid forming enzymes in the stomach wall. The output of the stomach acid is reduced by blocking these enzymes and thus the stomach can be healed. Omeprazole was the reference anti-ulcer treatment in this sample and was extracted from (happy pharmacy) in Erbil city. The medication was supplied by mouth to rats at 20mg/kg body weight suspended in distilled water (5ml/kg) (Pedernera *et al*., 2006).

3.6 Induction of Gastritis

Gastritis was induced by feeding rats with bacterium *H. pylori* that was collected from biopsy samples from clinical patient. The rats were feed on basal diet for one week in the animal house of the previous research center for adaptation. In this study the rats were inoculated by gavage with 1ml *H. pylori* suspension (5×10^{8}-5×10^{10} CFU/mL) twice daily at an interval of 4h for seven consecutive days. The time required infect the rat with *H. pylori* was determined by *H. pylori* antibody test every day after complete seven days inoculated by *H. pylori* (McColl, 2010).

3.7 Collection of *Helicobacter pylori* Samples

These work, was performed between June 2020 and August 2020 in the Kalar Hospital (a district of Sulaymaniah city in the east part of the Iraqi Kurdistan Region). Patients with symptoms of digestion, upper abdominal pain and vomiting, as tissue biopsy samples were collected by a pathologist of the digestive system, after the patients agreed to take samples, two biopsies were collected from each patient to avoid patchy distribution from two different Antrum sites the preferred location of the bacterium *H. pylori* to avoid the acid-secreting parietal cells, the *H. pylori* sample collection were decide according to (Cheng et al., 2015) that collect *H. pylori* samples from patient.

3.7.1 Columbia Blood Agar Medium

Following the manufacturer's instructions, the medium was prepared by dissolving 39g of the agar in 1liter of distilled water, leaving the medium for sterilization in the autoclave. The media was cooled to 45ºC and added 7% of sheep blood (70 ml/L). Then 5ml of the earlier prepared antibiotic are added. The medium is distributed in sterile petri dishes at a rate of 20ml for each petri dish, and after solidification of the medium, it is kept at a temperature of -4ºC. After modifying antibiotic method, 10mg of antibiotic was dissolved Vancomycin in 5ml of distilled water and sterilize the final solution with fine membrane filters according to (Dent and McNulty, 1988).

3.7.2 Cultivation of specimens

The (Parsonnet et al., 1988) method was used to culture samples as following:

1. After the clinical samples arrive at the laboratory and within a period not exceeding two hours, the tissue biopsy samples intended to isolate the bacteria were crushed and homogenized with sterile wooden sticks that were well crushed until the tissue turned into a homogeneous mixture.
2. The mixture inoculated by using the loop in the center of Colombia agar by streaking method at a rate of two repetitions per dish.
3. Petri dishes were transfer in to (Anaerobic Jar-Oxoid) container that containing moisturized cotton with sterile distilled water to provide the required humidity, then added the gas generation (Microaerophilic) Kit, closed the container tightly to provide the required conditions. Put the anaerobic receptacle in the incubator at 37°C for 3-14 days. After incubation period the bacteria diagnosed by biochemical test and other approved diagnostic methods.
4. The isolates were stored on a broth containing broth BHI 20% glycerol for a period of (24-48 hours) and after that it was stored at a temperature of (-20°C).

3.7.3 Microaerophilic Atmosphere

The under-ventilated conditions were provided by the use of a kit generating gas located directly in the anaerobic container containing the cultivated dishes and close the container tightly to provide the necessary gaseous conditions (5% O_2 -10% CO_2 & 85% N_2), then put the airtight anaerobic vessel in the incubator at a temperature of 37°C for a period of (3-14) days after the end of the incubation period. Bacteria developing in culture media were observed and diagnosed with bacteriology and biochemically according to the approved diagnostic criteria (Holloway et al., 1994).

3.8 Identification of Isolates

3.8.1 Diagnosis of *H. pylori*

The colonies of *H. pylori* were phenotypically identified on the basis of colony shape and arrangement with examined under a light microscope, and based on a set of diagnostic tests in detection of its existence (Holt, 1994).

3.8.2 *H. pylori* bacterial colonies appearance

The colonies grow sporadically and far apart in the middle of Colombia agar is fortified with growth factors and is round, small in size, colorless, transparent or grayish, somewhat convex, with a mucous structure resembling a drop of water in the middle.

3.8.3 Catalase test

On a clean glass slide, a drop of hydrogen peroxide H_2O_2 was placed and a portion of the growing bacterial colony was transferred to the center of the glass slide by a sterile wood stick and mixed with the drop of H_2O_2 (Tadesse and Alem, 2006).

3.8.4 Oxidase test

Drops of the oxidase enzyme reagent were placed on a filter paper or sterile cotton, and then a portion of a growing colony was transferred to the agar medium fed by a sterile wood stick and mix with the reagent drop (Forbes et al., 2016).

3.8.5 Gram stain solution

Use the traditional gram stain method, by changing safranin with carbol Fuchsin (0.75%) to dye fixed, gram-stained and colonized swabs taken from colonies developing on solid culture media for the purpose of diagnosis (Cruickshank, 1975).

3.9 Experimental Design

Forty eight male albino rats were used for the experiment, Rats body weight ranged from 160±20 gram 7-9 week old, the rats randomly allocated to four groups, each group of twelve rats were purchased from Zakho University, housed in well aerated cages under hygienic condition. The rats were fed on basal diet for one week in the animal house of the College of Education in the Sallahadin University-Erbil. In this study the rats were fasted in the morning and afternoon first inoculated with 1ml *H. pylori* suspension (5×10^8-5×10^{10} CFU/ml) by gavage twice daily at an interval of 4h for seven consecutive days then immediately inoculated with plant extracts, then left on normal feeding for 15 days to seen the effect of the plants extracts with *H. pylori* activity (McColl, 2010), before inoculating animals with plants extract and *H. pylori* the animals done the *H. pylori* test

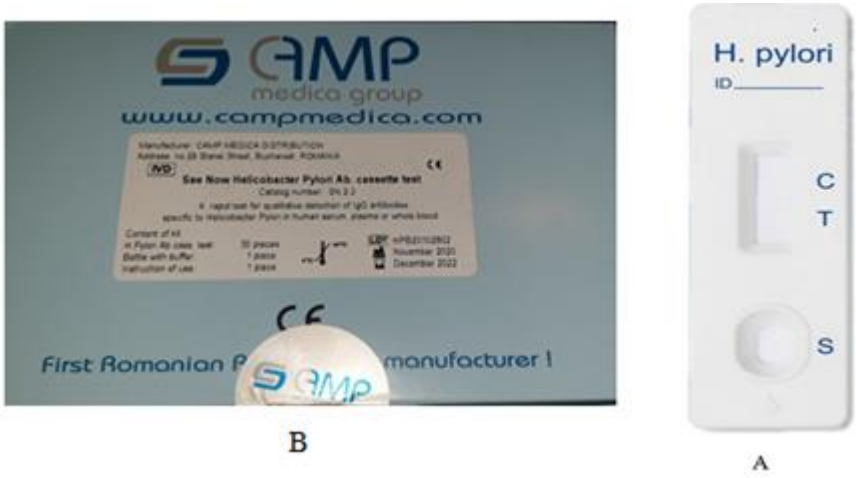
via *H. pylori* antibody test to ensure that did not infected with *H. pylori* before, that showed the test in **(Figure 3.1)**.

Group 1: Gastritis, Control positive (rats infected with *H. pylori*).

Group 2: This group infected with *H. pylori*, six rats received high dose (500mg/kg body weight) and low dose (250mg/kg body weight) of aqueous extract *Hypericum perforatum* orally at the concentration of (5ml/kg body weight) for three days (AlRashdi et al., 2012).

Group 3: This group infected with *H. pylori*, six rats received high dose (500mg/kg body weight) and low dose (250mg/kg body weight) of aqueous extract *Punica granatum* orally at the concentration of (5ml/kg body weight**)** for three days.

Group 4: This group infected with *H. pylori*, twelve rats received (20mg/kg) of omeprazole for three days. (AlRashdi *et al*., 2012).



3.1 Rapid H. pylori antibody test kit

3.9.1 Preparation and Selection of Doses of plant extract

The plant extraction preparation (Odabaş-Serin et al., 2020) and selection the doses were decided according to the (Chen et al., 2018).

3.10 Experimental Procedure

3.10.1 Sample Collection

In last period of prevention, anesthetized of the rats done by intramuscular injection of mixed xylazine-ketamine (1:9) as a single dose in the same syringe, The Stomach was removed of each rat from each group, then dissects the stomach and preserved the liquid inside stomach to calculate the pH level.

3.10.2 Gross gastric lesions evaluation

Ulcers located in the gastric mucosa, parallel to the long stomach axis, emerged as elongated bands of hemorrhagic lesions. Thus the damage was studied in each gastric mucosa specimen. A calipers (10 to 10 mm 2 = ulcer area) under the dissecting microscope measured the length of the stomach mucosa ulcer (mm) and the distance (mm) (1.8x). By counting the number of small squares, 2 × 2mm, covering the length and width of each ulcer band, the area of each ulcer lesion was estimated. In the measurement of the ulcer region (UA), the total of the areas of all lesions for each stomach was added, wherein the sum of small squares × 4 × 1.8 = UA mm2 as previously defined by Kauffman and Grossman (1978) with minor adjustment. The inhibition percentage (I %) was calculated by the following formula as described by Njar et al., (1995) with slight modification. (I %) = [(UA control – UA treated) ÷ UA control] × 100%.

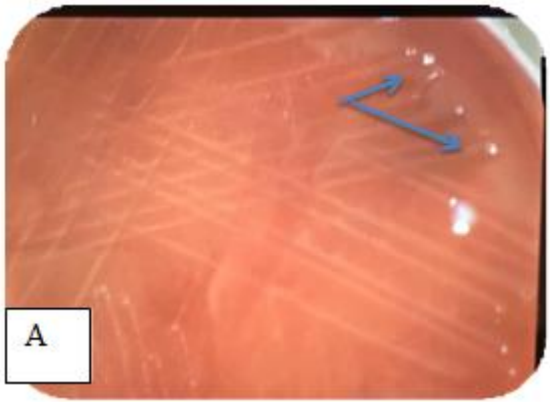
3.10.3 Measurement and evaluation of acid content of gastric juice (pH)

Gastric material samples were tested for hydrogen ion accumulation by pH metric titration using a digital pH metre with 0.1N NaOH solutions. For studying the effect of *H. perforatum*, *P. granatum*, and omeprazole on gastritis in rat, the pH level of stomach was individually recorded for evaluation the pH level of all groups of rats to know the effect and correlation of plant extract with pH level.

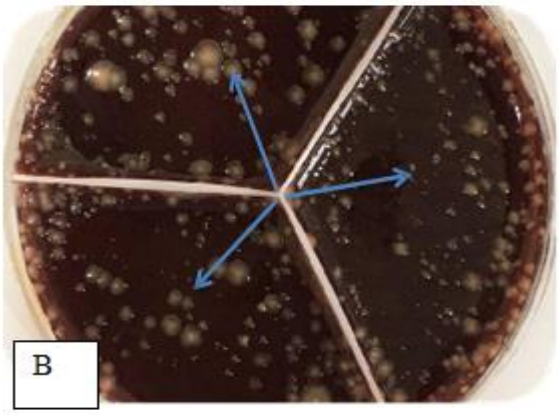
4 RESULTS

4.1 Phenotypic properties

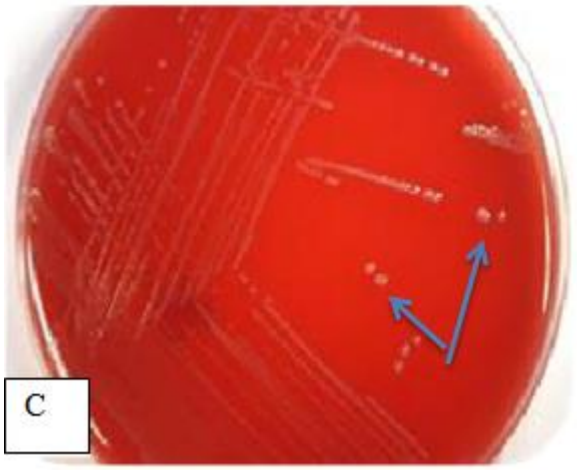
H. pylori colonies appeared after an incubation period (3-14) days in under-ventilated conditions while providing high humidity at a temperature of 37°C (Goodwin et al., 1998), as shown in the following Figures 4.1 (A.B.C.D):



(a)



(b)



(c)

Figure 4.1 *H. pylori* colonies growthing on solid media.

Figure (4.1 a) show the colonies grow on the Columbia media agar for a period of (14) days at a temperature of 37°C. Figure (4.1 b) Colonies growing on the Columbia media agar for (7) days at a temperature of 37°C. Figure (4.1 c) Colonies growing on the blood media agar for (72) hours at a temperature of 37°C.

Colonies developing on selective media were identified as bacterium *H. pylori* dependence shaped in culture medium, stained with gram stain, and positive colonies for enzyme testing urease, catalase and oxidase, after cultivation of tissue biopsies on the selective culture medium (3-14) day incubation in under-ventilated conditions using gas saving bags (30%) isolation of *H. pylori*. The colonies appeared as small cells with convex circular edges and transparent, similar to a drop of water, or gray in color, as in Figure 4.1. After getting colonies Examined under a microscope with a gram stain, it appeared as bacillary gram-negative cells a spiral shape appears in the form of the wing of a seagull, *H. Pylori* bacteria were isolated to the results of bacterial culture based on 30%, Test outputs cultivation and the result of its comparison with the RUT test found a significant difference, meaning that the results of the culture test had no effect Valuable for diagnosis, This test's low detection efficiency is due to the nature of the glutton bacteria and sensitive to both heat and addition to that the difficulty of survival of these bacteria alive at room temperature during the period of sample collection and competition with normal gastric flora, it plays a major role in the isolate inhibition.

4.2 Biochemical tests

All isolates obtained after growing in low ventilated conditions gave a result positive for the catalase enzyme test, which appears in bubbles as in Figure (4.2), Also the oxidase enzyme that appears in purple (Figure 4.3) and this result is agreed with the diagnostic description of (Brooks et al., 2004).

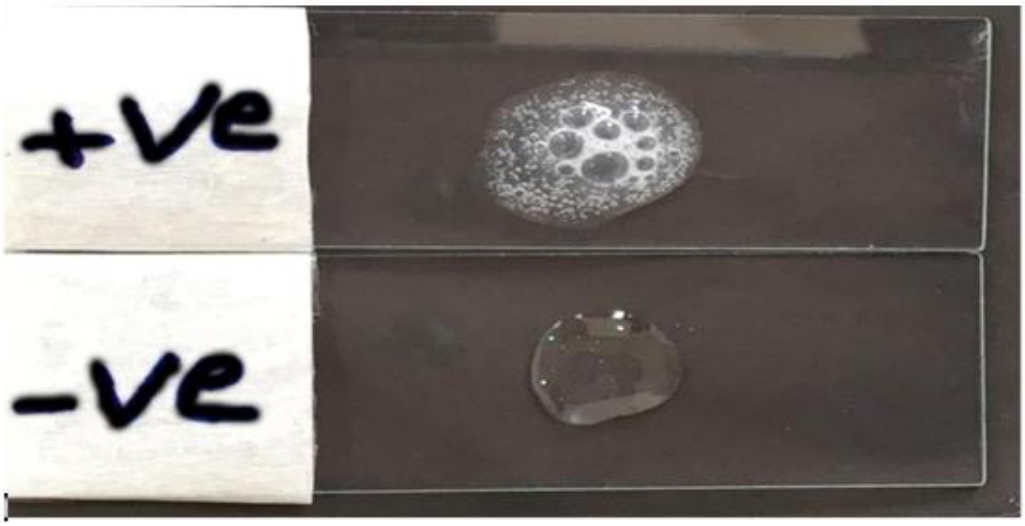


Figure 4.2 The results of the catalase enzyme test.

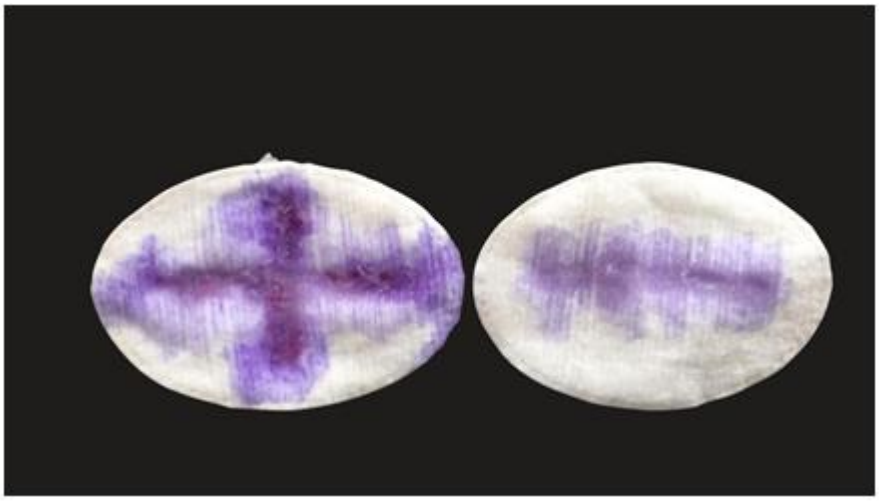
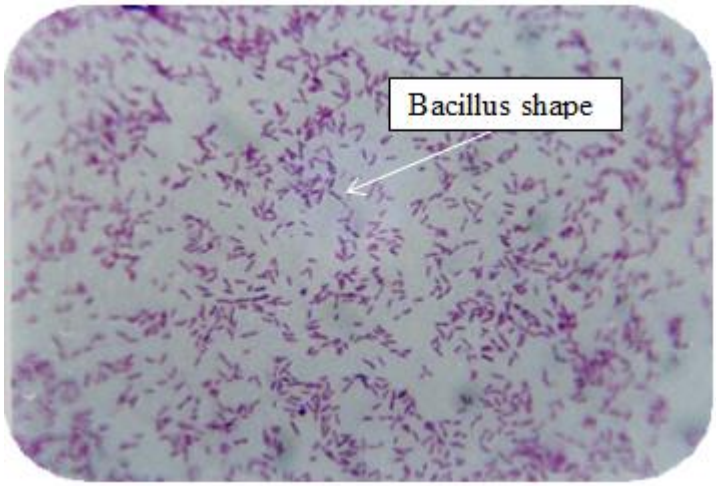


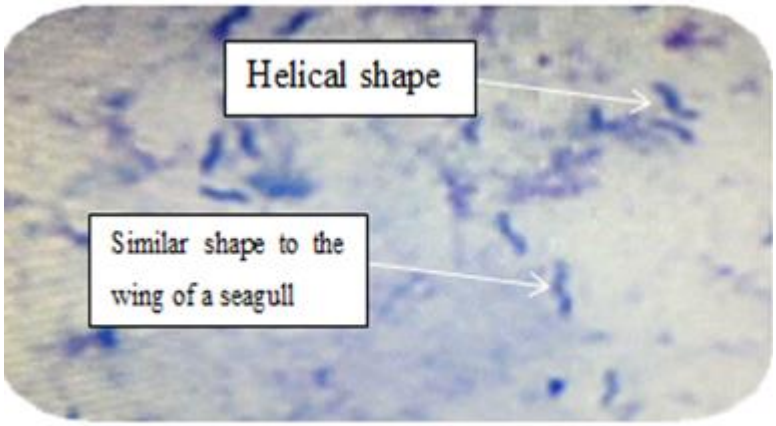
Figure 4.3 The results of the oxidase enzyme test.

4.2.1 Microscopic examination

Figures (4.4a, b) gram stained *H. pylori* colonies the presence of heterozygous shapes when examined microscopically; its colonies appeared similar to the letter S and in a baton-like shape in the form of a seagull's wing.



(a)



(b)¶

Figure 4.4 The shape of the Gram-stained H. pylori cells.

Where form of Gram-stained bacterial *H. pylori* cells colonies under 40X power as shown in Figure 4.4a. Figure (4.4b) presented the Form of Gram-stained bacterial H. pylori cells colonies under 100X power.

4.3 Effect of Aqueous Extract *Hypericum perforatum*

4.3.1 Gross evaluation of gastric lesion

Table (4.3) shown the antiulcer activity of aqueous extract *H. perforatum* against gastric lesion model caused by *H. pylori*. Compared with ulcer control group, results showed that rat's stomach prevented with high dose and low dose of *H. perforatum* extracts with being given *H. pylori* solution reduced areas of gastric ulcer formation.

H. pylori solution produced extensive visible black hemorrhagic lesions of gastric mucosa. Moreover, this plant extract have a significant effect of formation of the ulcers in rats stomach prevented with the high dose extract of this plant (500mg/kg) at inhibition rate (50.65%). The gastric ulcer in rats prevented with low dose *H. perforatum* extract (250mg/kg) at inhibition rate (24.97%) was comparable with control group which is in Figures (4.6), the length and width of each lesion were measured and the sum of the area of all lesions for each stomach was expressed as the ulcer area (mm^2). The ulcer area (UA) was calculated as described by Kauffman and Grossman (1978). The inhibition percentage (I.0%) was calculated using the formula (I %) = [(UA control – UA treated) ÷ UA control] × 100%.

Table 4.1 Shown cooperation of ulcer area and ulcer inhibition between ulcer control group and H. perforatum

Animal group	Prevention (5ml/kg) dose	Ulcer Area (mm^2) Mean ± SEM	Ulcer Inhibition %
1	Ulcer control group	815.00	0
2	High dose *H. perforatum*	402.20	50.65
	Low dose *H. perforatum*	611.45	24.97

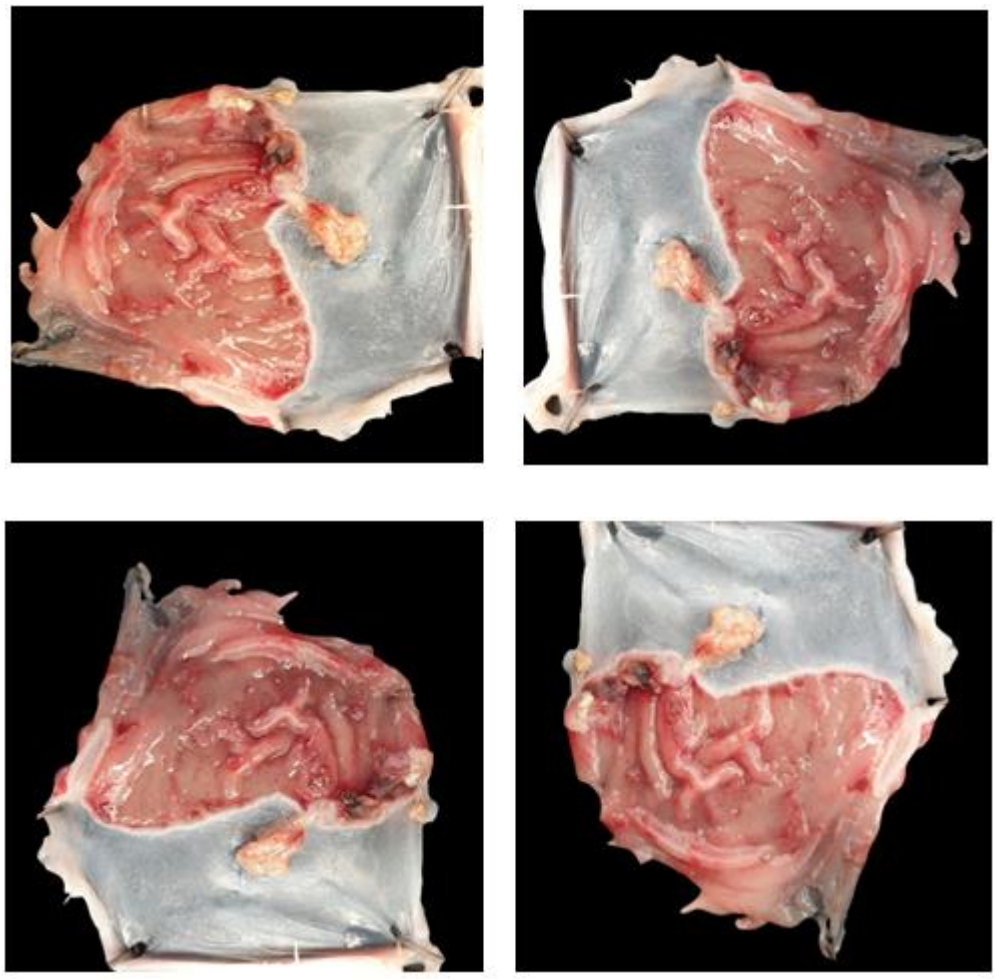


Figure 4.5 Dissect stomach of rats treated with *H. perforatum* extract.

4.3.2 pH of gastric content in *Hypericum perforatum* aqueous extract

The pH of the stomach is one of the parameters that considered in the study, The acidity of gastric content in experimental animals prevented with *H. perforatum* was increased compared with that of the ulcer control group, The *H. perforatum* showed that the pH was between (4-4.5), but the low dose was (3.5-4) but the control group showed (3.5), figure (4.7).

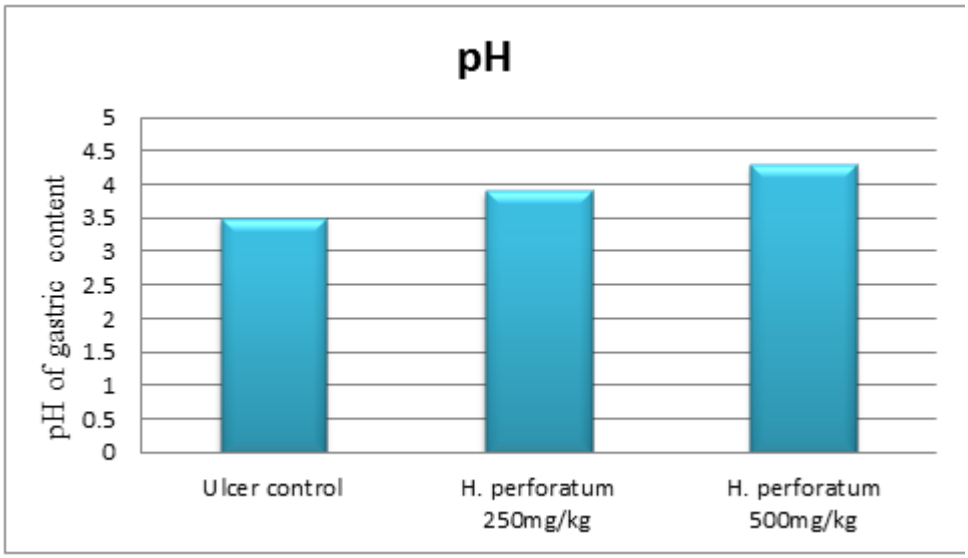
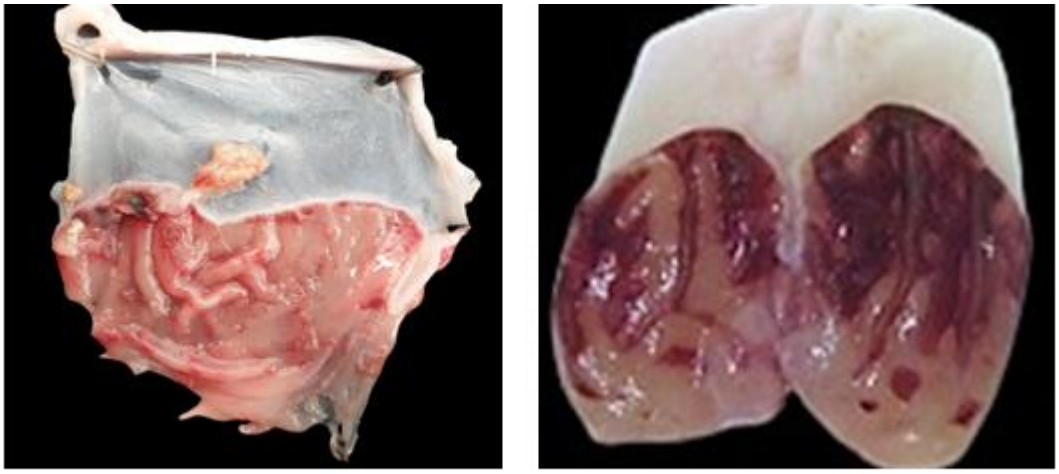


Figure 4.6 Effect of *Hypericum perforatum* on pH level of gastric content

4.3.3 Stomach color change in rats prevented with *Hypericum perforatum* aqueous extract

The color of the stomach is one of the parameters that considered in the study, The color of prevented rat stomach with *H. perforatum* are changed comparing with ulcer control group, white bright color was found in the stomach of the rats with *H. perforatum*, pink to red color was found at the each of the stomach, otherwise the control groups stomach was totally dark red because of the ulcer and inflammation that caused by *H. pylori* (Figure 4.8).



(a) (b)

Figure 4.7 Rats stomach color change Cooperation in (a) *H. perforatum* extract, (b) Control group

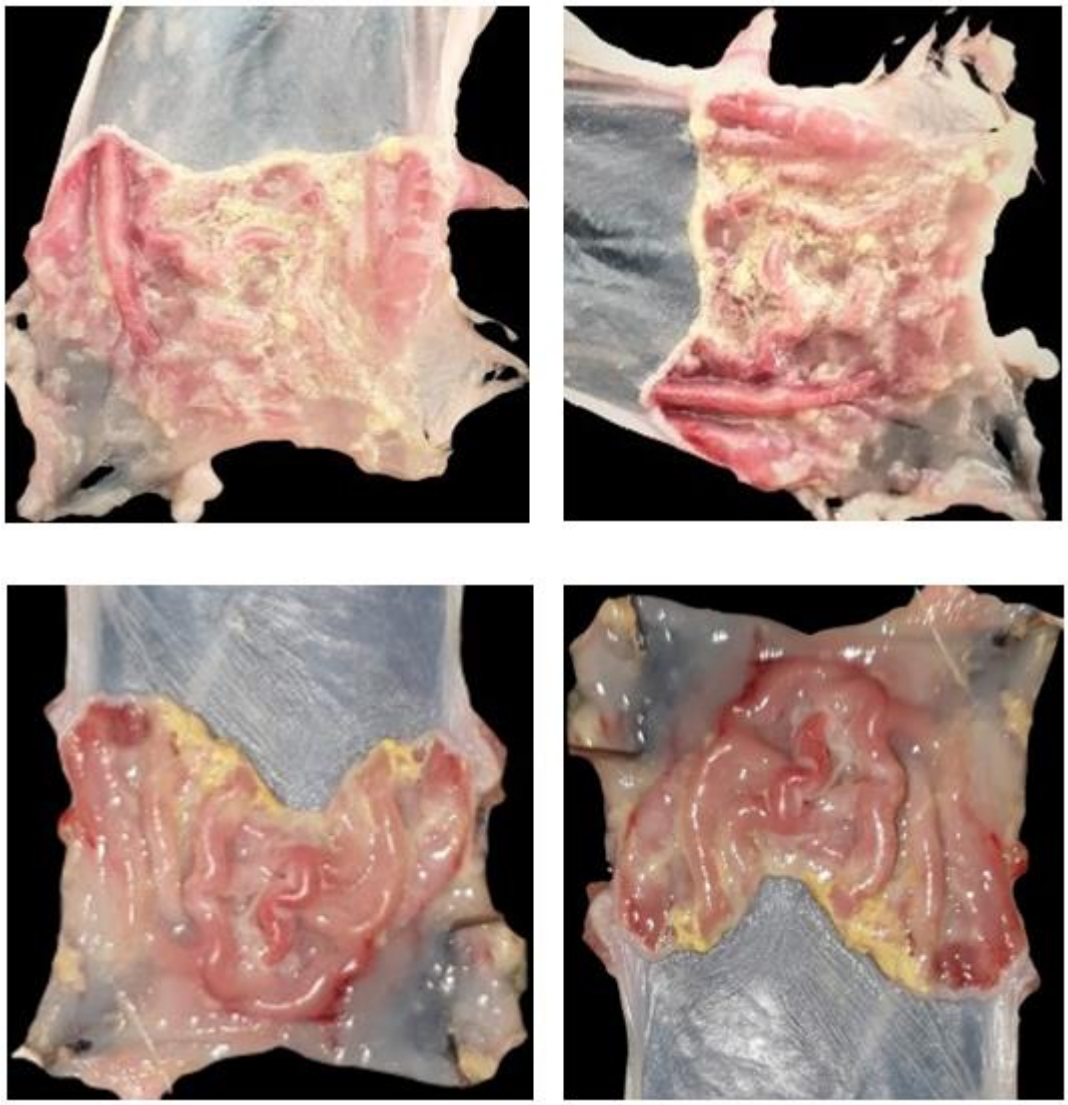
4.4 Effect of *Punica granatum* Aqueous Extract

4.4.1 Gross evaluation of gastric lesion

Group 1 participants, ulcer control animals, demonstrated serious mucosal damage with ulcer region in ulcer prevention studies (815.00mm2) Table (4.4). A significant decrease in the ulcer area was observed in third group animals. For the groups prevented with the *Punica granatum* aqueous extracts, the ulcer area was significantly decreased in a dose-dependent manner when compared to group1. The ulcer area was significantly reduced from 815.00mm^2 (control rats) to (125.50 mm^2) in the rats treated with high dose 500mg/kg of high *P. granatum* extract. As shown in (Table 4.4), the rats stomach prevented with high dose 500mg/kg of *P. granatum* extract exhibited the highest inhibition percentage of ulcer area formation which was (84.60%), *P. granatum* extracts of low dose 250mg/kg (42.87%), The high dose 500mg/kg of extract *P. granatum* prior to gastric ulcer treatment was effective in ulcer prevention.

Table 4.2 Effect of aqueous extract of *Punica granatum* on different gastric parameters of *H. pylori* induced rats' gastric ulcer.

Animal group	Prevention (5ml/kg) dose	Ulcer Area (mm^2) Mean ± SEM	Ulcer Inhibition %
1	Ulcer control group	815.00	0
3	High dose *Punica granatum*	125.50	84.60
	Low dose *Punica granatum*	465.60	42.87



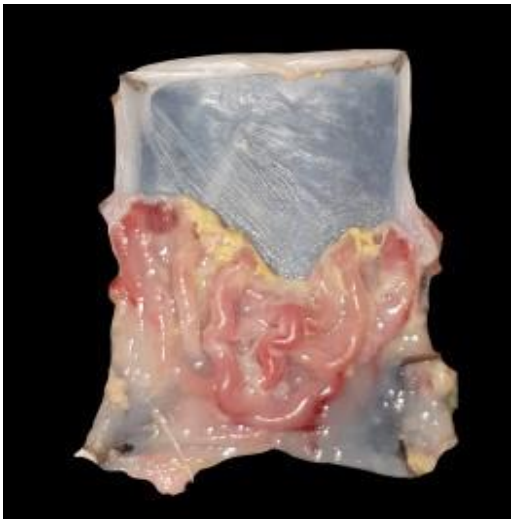


Figure 4.8 Shown the rats stomach prevented with *Punica granatum* extract

4.4.2 pH of gastric content in *Punica granatum* aqueous extract

The pH of the stomach is one of the parameters that considered in the study, The acidity of gastric content in experimental animals prevented with *P. granatum* was increased significantly compared with that of the ulcer control group, The *P. granatum* showed that the pH ranged between (6-6.5), and the low dose limited between (4.5–5), beside of that, the control group gained (3.5), Figure (4.10).

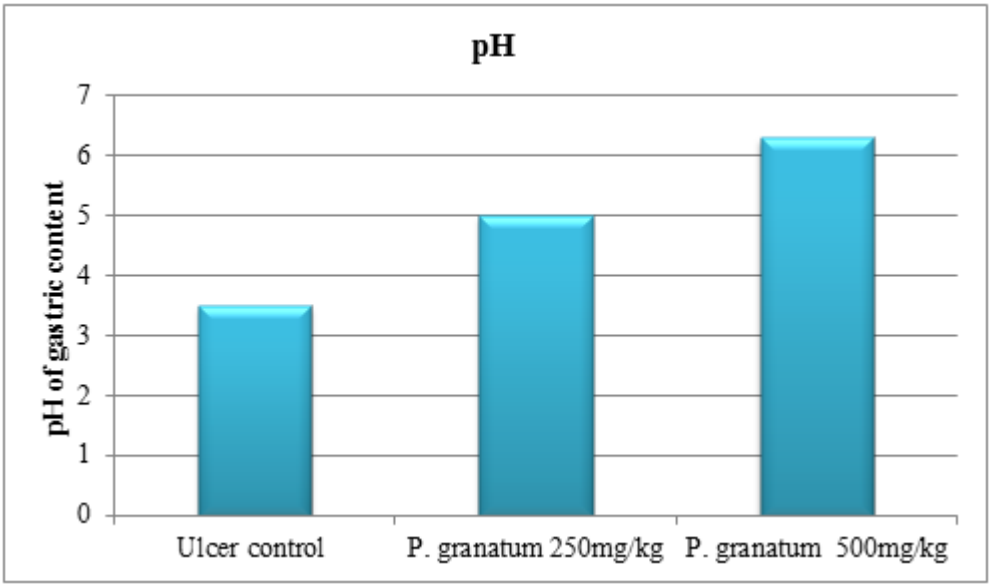


Figure 4.9 Effect of Punica granatum extract on pH of gastric content

4.4.3 Stomach color change in rats treated with *Punica granatum* aqueous extract

The color of the stomach is one of the parameters that considered in the study, The color of prevented rat stomach with *P. granatum* are significantly changed comparing with ulcer control group, white bright color was found in the stomach of the rats with *P. granatum*, white to pink color was found at the each of the stomach, otherwise the control groups stomach was totally dark red because of the ulcer and inflammation that caused by *H. pylori* (Figure 4.11).

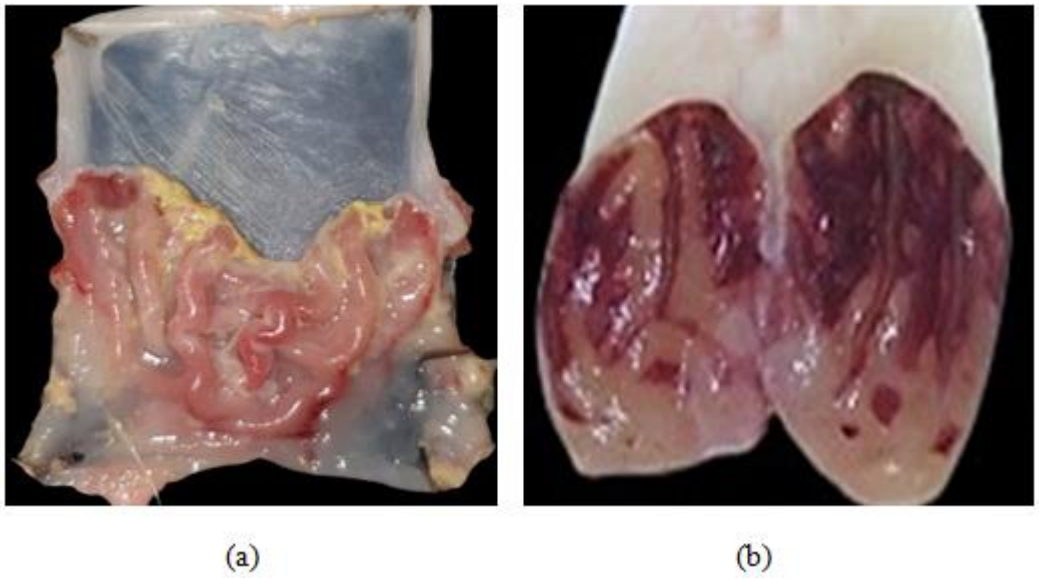


Figure 4.10 Rats stomach color change Cooperation in (a) *Punica granatum* extract, (b) Control group.

4.5 Effect of Antibacterial Omeprazole

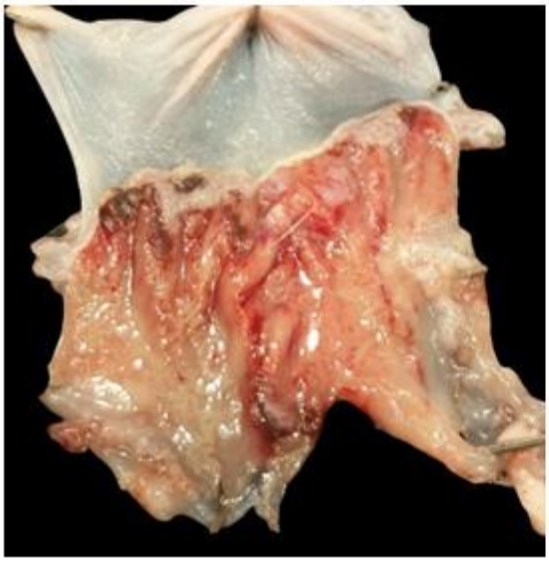
4.5.1 Gross evaluation of gastric lesion

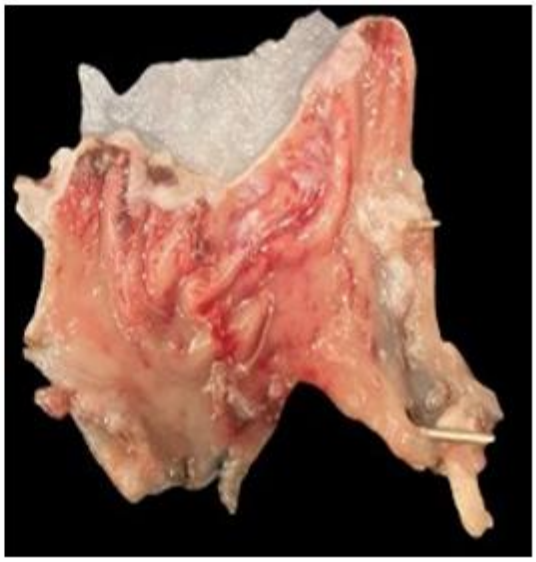
The antiulcer activity of omeprazole drug *H. pylori* induced gastric lesion model is shown in Table 4.5. Results showed that rat's stomach prevented with omeprazole with being given *H. pylori* solution reduced areas of gastric ulcer formation at rate (24.50%) compared with ulcer control group. *H. pylori* aqueous solution produced extensive visible

black hemorrhagic lesions of gastric mucosa. The significant inhibition of gastric ulcer in rats prevented with omeprazole was comparable with control group.

Table 4.3 Shown cooperation of ulcer area and ulcer inhibition between ulcer control group and omeprazole antibiotic drug

Animal group	Prevention (5ml/kg) dose	Ulcer Area (mm^2) Mean ± SEM	Ulcer Inhibition %
1	Ulcer control group	815.00	0
4	Omeprazole	615.25	24.50





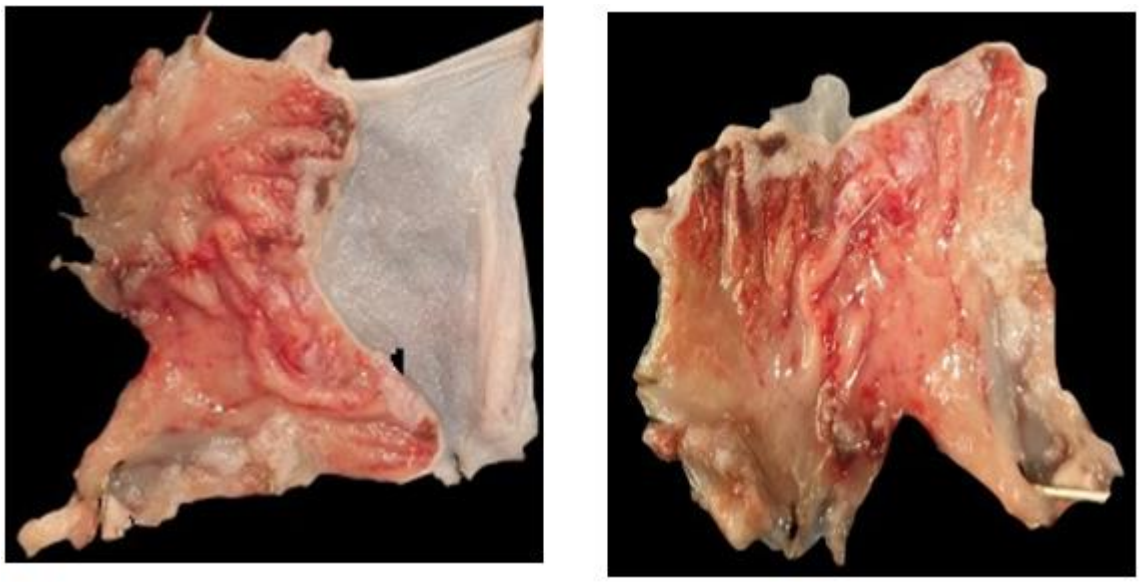


Figure 4.11 Shown the rats stomach prevented with omeprazole

4.5.2 pH of gastric content in omeprazole

The pH of the stomach is one of the parameters that considered in the study, The acidity of gastric content in experimental animals prevented with omeprazole was increased compared with that of the ulcer control group, The omeprazole showed that the pH was between (5-5.5), but the control group showed (3.5), Figure (4.13).

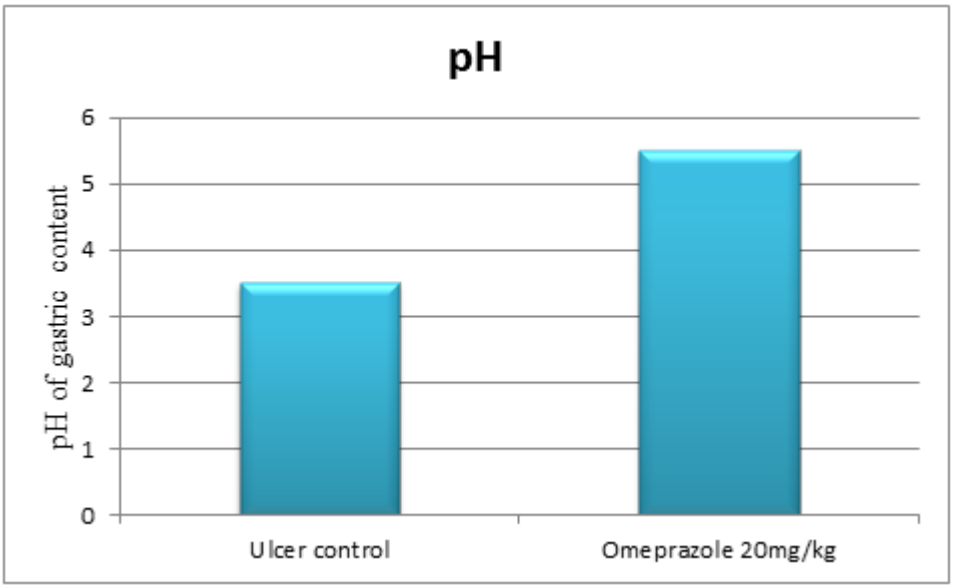


Figure 4.12 Effect of Omeprazole antibiotic drugs on pH of gastric content

4.5.3 Stomach color change in rats prevented with omeprazole

The color of the stomach is one of the parameters that considered in the study, The color of prevented rat stomach with omeprazole are changed comparing with ulcer control group, red bright color was found in the stomach of the rats with omeprazole, pink to red color was found at the each of the stomach, otherwise the control groups stomach was totally dark red because of the ulcer and inflammation that caused by *H. pylori* as presented in Figure 4.14.

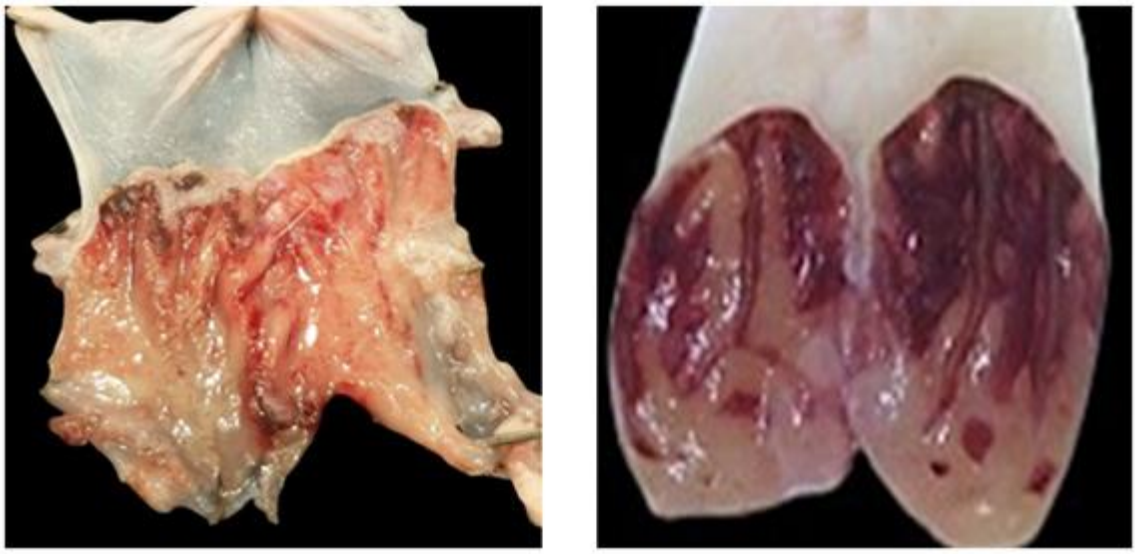


Figure 4.13 Rats stomach color change Cooperation in (a) omeprazole, (b) Control group

4.6 Effect of Aqueous Extract of *Hypericum perforatum*, *Punica granatum* and antibacterial omeprazole

The antiulcer activity of aqueous extract *H. perforatum* against gastric lesion model caused by *H. pylori* is reported in Table 4.6. Results showed that ulcer area of rat's stomach with high dose (500mg/kg) of *H. perforatum* extracts was inhibited at rate (50.65%) gastric ulcer formation compared with ulcer control group.

A significant decrease in the ulcer area was observed in third group animals prevented with the *P. granatum* aqueous extracts, the ulcer area was significantly decreased in a dose-dependent manner when compared to control group. The ulcer area was significantly reduced or inhibited from (815.00mm^2 control group rats) to (125.50 mm^2) exhibited the

highest inhibition percentage of ulcer area formation which was (84.60%) in the rats prevented with high dose (500mg/kg) of *P. granatum* extract as shown in Table 4.6.

The antiulcer activity of omeprazole drug *H. pylori* induced gastric lesion model is shown in Table 4.6, Results showed that rats' stomach prevented with omeprazole with being given *H. pylori* solution reduced areas of gastric ulcer formation at rate (24.50%) ulcer area inhibition compared with gastric ulcer control group.

Animal group	Prevention (5ml/kg) dose	Ulcer Area (mm^2) Mean ± SEM	Ulcer Inhibition %
1	Ulcer control group	815.00	0
2	High dose *H. perforatum*	402.20	50.65
	Low dose *H. perforatum*	611.45	24.97
3	High dose *P. granatum*	125.50	84.60
	Low dose *P. granatum*	465.60	42.87
4	Omeprazole	615.25	24.50

Table 4.4 Effect of Aqueous Extract of *Hypericum perforatum*, *Punica granatum* and antibacterial Omeprazole of gastric ulcer production and inhibition the ulcer

4.7 Evaluation of pH level of gastric content between *Hypericum perforatum*, *Punica granatum* and antibacterial Omeprazole

The pH of the stomach is one of the parameters that considered in the study, The acidity of gastric content in experimental animals prevented with *H. perforatum* was increased compared with that of the ulcer control group, The *H. perforatum* showed that the pH was between (4-4.5), while the acidity of gastric content in experimental animals prevented with *P. granatum* extract was increased significantly compared with that of the ulcer control group, The *P. granatum* showed that the pH level was between (6-6.5), although the acidity of gastric content in experimental animals prevented with omeprazole

was increased compared with the ulcer control group, omeprazole showed that the pH was between (5-5.5) however control group pH level was at rate (3.5).

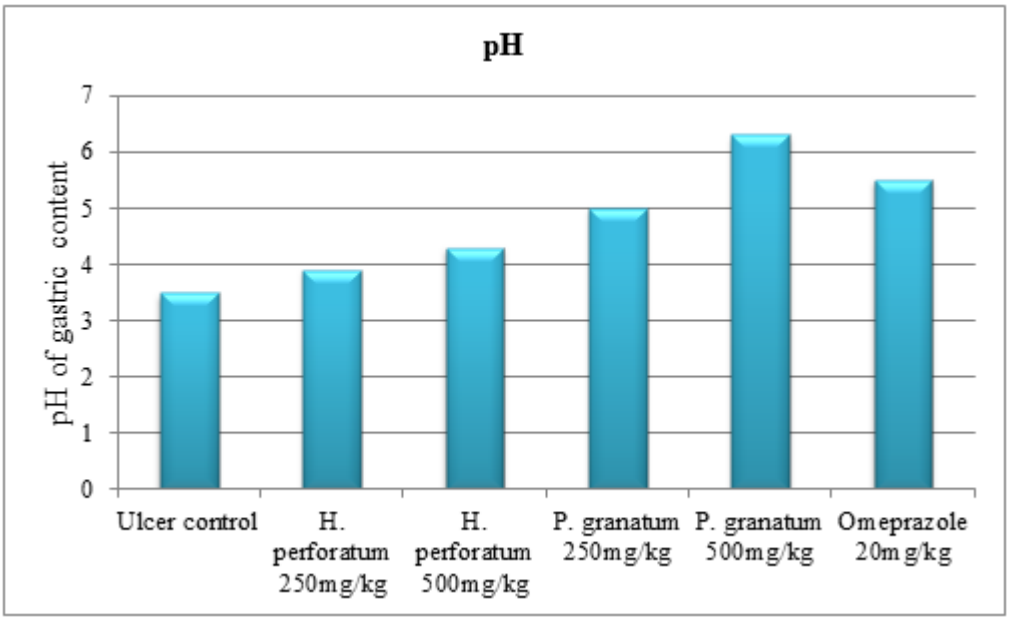


Figure 4.14 evaluation of pH level of gastric content between *Hypericum perforatum, Punica granatum* and antibacterial Omeprazole

4.8 Evaluation of stomach color change in rats between *Hypericum perforatum, Punica granatum* and antibacterial Omeprazole

The color of the stomach is one of the parameters that considered in the study, color of prevented rat stomach with *H. perforatum* are significantly changed comparing with ulcer control group, white bright color was found in the stomach of the rats with *H. perforatum*, pink to red color was found at the each of the stomach, otherwise the control groups stomach was totally dark red because of the ulcer and inflammation that caused by *H. pylori*, however the color of prevented rat stomach with *P. granatum* are significantly changed comparing with ulcer control group, white bright color was found in the stomach of the rats with *P. granatum*, white to pink color was found at the each of the stomach, otherwise the control groups stomach was totally dark red, while the color of prevented rat stomach with omeprazole are changed comparing with ulcer control group, red bright color was found in the stomach of the rats with omeprazole.

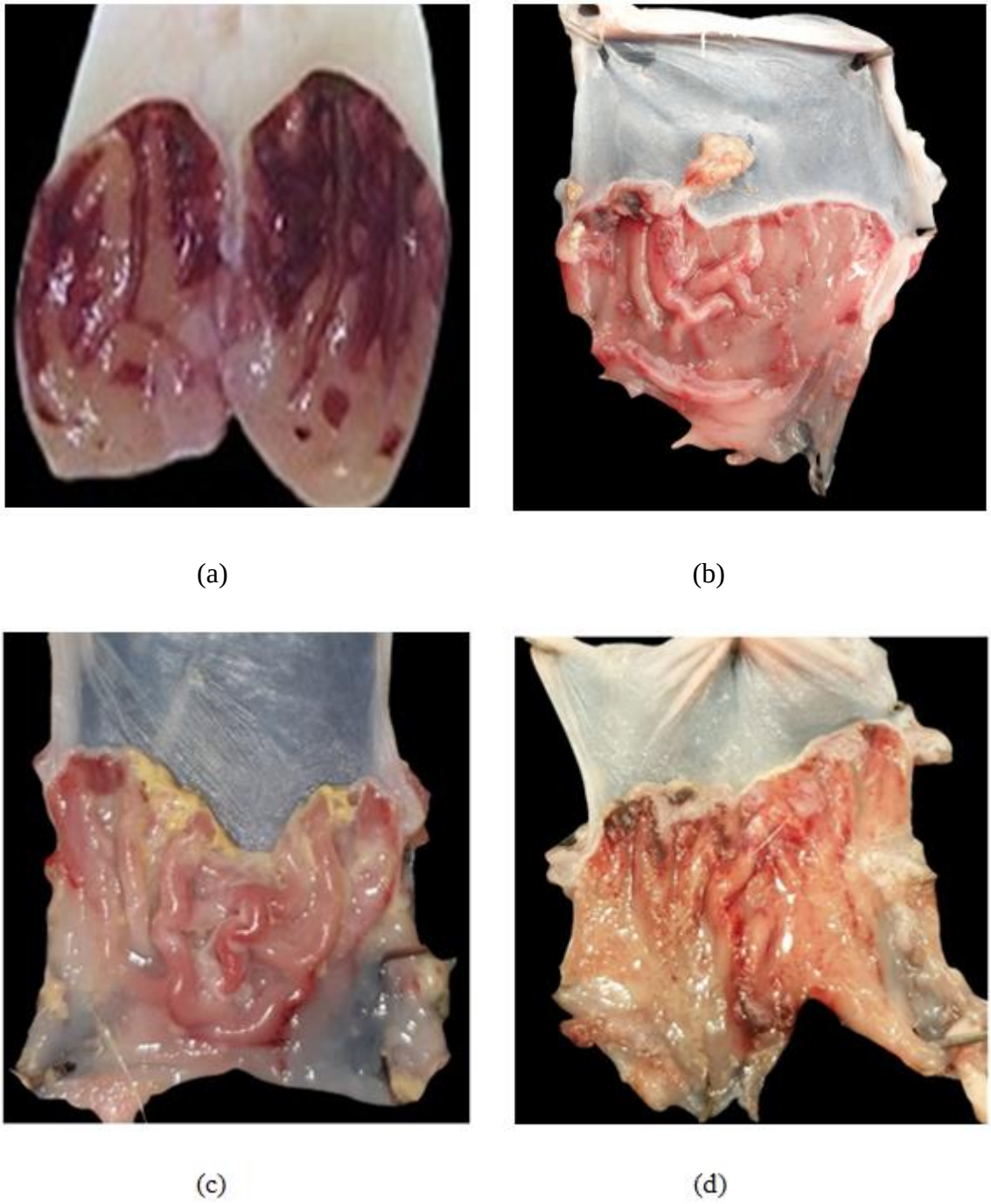


Figure 4.15 (a) shown the rats stomach control group, (b) rats stomach prevented with *H. perforatum* ectract, (c) rats stomach prevented with *P. granatum* extract, (d) rats stomach prevented with antibacterial omeprazole

4.9 Microbiological test before inoculating with *H.pylori* and plants extract

All rats groups was tested via *H. pylori* antibody test strip to ensure that all rats was not infected with *H. pylori* before starting our practical section in inoculating with plants extract and *H. pylori*, the result was none of the rats in all groups infected with *H. pylori* before.

5 DISCUSSION

H. pylori which induces a group of diseases like (gastric ulcer, gastric cancer, peptic ulcer, gastritis), Nowadays, infections with. *H. pylori* are a common infectious disease. *H. pylori* damage the gastric mucosa, usually in the form of peptic ulcer disease or even gastric malignancies. Gastritis is one of the most prominent clinical symptoms of *Helicobacter pylori* infection. The presence of inflammation of the gastric mucosa or sub mucosa caused by Helicobacter pylori leads to moderate to severe histological transformation.

The extent of gastritis is correlated with the density of *H. pylori*, damage to the gastric mucosa and inflammation (Yulizal et al., 2020).

Gastric ulcers are a recurring condition where the mucosa lining of the stomach breaks down. There is a physiological equilibrium between offensive factors and the preservation of the mucosa. When this equilibrium is compromised in favor of aggressive variables, gastric mucosa damage happens. *H. pylori*, non-steroidal anti-inflammatory medications, ethanol and genetic factors are some of the violent variables. As a consequence of its use to cause gastric ulcers in animal models, ethanol has a devastating effect on gastric mucosa (Ofusori et al., 2019).

After the first examination through the endoscopy of the endoscopy unit patients according to the result of diagnostic test (culture and rapid urease test) RUT test is the invasive methods of discovery for *H. pylori* and it is less expensive than other invasive biopsy-based diagnostic methods with very good specificity.

The diagnostic methods used in the current study showed variation in detection rates bacteria and a countdown of detection methods according to percentages in identifying samples positive results, the rapid urease test showed the highest rate in diagnosing of *H. pylori* at 70%, this test mainly depends on the number of bacteria in a tissue biopsy is a specific test because it depends on the ability of the bacteria itself to produce an enzyme

urease in urea medium the routine culture test for histopathology also showed positive result 30%, Different results shown by the another study that done by (Ibrahim, et at., 2013) “the rapid urease test showed, specificity of 100% and sensitivity of 88.9%, negative predictive value of 88.5% and positive predictive value of 100% on testing (27) positive rapid fecal tests”.

However, current research findings were inconsistent with (Choi et al., 2012) the results of studies carried out in individuals with an RUT insulation average of 96.7 percent, in cultivation 68.9 percent, in the cultures diagnosed result in the above study positive in (75) patients at rate (30.4%), and negative in (171) of the 246 patients at rate (69.6 percent) with *H. pylori* inflammation. The rapid urease test taken biopsy in the antrum negative in 175 patients at rate (71.1%), positive in (71) patients at rate (28.9%) shown the results were consistent in culture diagnosis method with our current study result, while did not agree with our current study result in rapid urease test method (Lee et al., 2013).

Result of current study shown that positive rate for the rapid urease test (70%) on the other hand (30%) gave a negative result, “current study results disagree with conducted by (Reddy et al., 2015)” indicated that the test for patients were positive for the rapid urease test are (58%). The proportions may differ from an error in taking the sample from the infections position, or the low density of bacteria in the area and small size of the sample, A biopsy can negatively affect the outcome or the likelihood of a false-positive result due to the presence of other microorganisms that can produce urease enzyme, such as (Pseudomonas & Protease spp (Mégraud et al., 2014) as well as false-positive result detection if the incubation period is extended more than 24 hours (Uotani and Graham, 2015) hence sensitivity the specificity of this test depends on the sample size and quality.

Another study result shown that herbal plant *H. perforatum* had antimicrobial affect or preventive effect against gastric ulcer disorder in the male albino rat animal model, That mostly caused by the bacterium *H. pylori*, our result were agree with (Reichling et al., 2001).

The present study agrees with their findings results shown that done by (Cayci and Dayioglu, 2009) with *H. perforatum* extract, gastric ulcer was prevented, generating substantial healing in an induced model of gastric ulcer, the study concluded to confirm

the arguments made by traditional medicine practitioners about the usefulness of the aerial part of *H. perforatum* for the treatment of ulcers.

"Numerous psychological and physical discomforts cause stomach ulcers in both humans and laboratory animals".

A defensive function against gastric ulcers is played by *H. perforatum*. The present findings suggest that its antiulcer influence is connected to increased secretion of adherent mucus and gastric pH content that may inhibit the production of free radicals extracted from oxygen and preserve normal MDA content, "the effects of anti-ulcers are associated with increased secretion of adhesive mucus and stomach pH content that can preserve normal MDA content and inhibit the production of free radicals extracted from oxygen", our result was accepted with the result finding in paperwork of inhibited gastric acid secretion of *H. perforatum* in pyloric-linked rats (Abdel-Salam, 2005).

H. pylori formed widely visible black hemorrhagic injuries on the stomach mucosa. Moreover this plant extraction had an effect at rate (50.65%) inhibition formation of stomach ulcers compared with control group in rats gastro-protective with the high dose plant extract of *H. perforatum* (500mg/kg) and 24.97% inhibition formation of the stomach ulcers in rats with low dose *H. perforatum* extract (250mg/kg), however another study result (Zdunić et al., 2009) were agrees with our study result finding that *H. perforatum* had effectiveness on gastro-protective in rat animal model.

Another study result shown that herbal plant *Punica granatum* had antimicrobial and anti-inflammatory affect or preventive effect against gastric ulcer disorder in the male albino rat animal model, That mostly caused by the bacterium *Helicobacter pylori*, our result were agree with (Prasad and Kunnaiah, 2014).

The present findings agrees with their findings results shown that done by (Moghaddam et al., 2013) gastric ulcer was prevented with *P. granatum* aqueous extract, produced significant healing in an induced gastric ulcer model. It was concluded that the present study supports the claims made by traditional medicine practitioners about the usefulness of parts of the *P. granatum* peel for gastric ulcer prevention.

Numerous psychological and physical stresses cause stomach ulcers in both humans and laboratory animals.

P. granatum acts as a protection against gastric ulcers. Current findings suggest that the anti-ulcer effect is associated with increased secretion of adherent mucus and increased pH of gastric contents, which can inhibit the production of oxygen-derived free radicals and keep the contents of MDA in a normal state. our result was agrees with result finding in (Alam et al., 2010) *P. granatum* prevents ulcers by increasing mucus secretion and pH level in pyloric ligation rats. Also shows anti-ulcer activity of AMP in experimentally induced gastric ulcer.

H. pylori formed widely visible black hemorrhagic injuries on the stomach mucosa, moreover plant extraction of pomegranate peel had an effect (84.60%) inhibition formation of stomach ulcers compared with control group in rats gastro-protective with the high dose plant extract of *P. granatum* (500mg/kg) and (42.87%) inhibition formation of the stomach ulcers in rats with low dose *P. granatum* extract (250mg/kg), however another study result (Gharzouli et al., 1999) were agrees with our study result finding that *P. granatum* play a major role on inhibition formation of stomach ulcers gastro-protective in rat animal model.

As an antibacterial agent, omeprazole, a standard drug used to treat gastric ulcers, plays a major role in preventing gastric ulcer disorders in a male albino rat animal model, that commonly caused by the *H. pylori*, According to the current study results, the stomach of mice treated with omeprazole infected with *H. pylori* showed a significant decrease in gastric ulcer formation sites at rate (24.50%) inhibition area compared to the ulcer control group. *H. pylori* solution produced widely visible black hemorrhagic lesions on the gastric mucosa.

Our study findings agrees with (Walan et al., 1989) that shown in 68 patients receiving concurrent non-steroidal anti-inflammatory drugs, the healing rates at four weeks were 61% in the group receiving 20 mg of omeprazole, significantly a great rate of patients in the omeprazole groups were free of symptoms and ulcers. At 4^{th} week haling rate was 61% in the group receiving omeprazole 20 mg, and a significant number of patients in the omeprazole group had no symptoms and ulcers.

Omeprazole acts as a protection against gastric ulcers. Our findings suggest that the anti-ulcer effect is associated with increased secretion of adherent mucus and increased pH of stomach contents which can inhibit the production of oxygen-derived free radicals

and keep the content of MDA normal. (AlRashdi et al., 2012) found a decrease in gastric acidity in treated animals. Omeprazole pretreatment can partially reduce the ulcer area and prevent stomach ulcers also has anti-secretory and protective effects.

H. pylori formed wide visible dark hemorrhagic lesions of stomach mucosa. Moreover, our study result shown that omeprazole drug produce (24.50%) inhibition formation of stomach ulcers compared with control group in rats. however another study (Wong et al., 2013) inhibition formation were (67.45%) disagrees with our study result in stomach ulcers gastro-protective in rats.

6 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The present study demonstrated that stomach organ was prevented from *H. pylori* induction of gastric ulcer by the herbal plants. Also it showed the prevention of stomach epithelium with aqueous extract of *Punica granatum*, *Hypericum perforatum*.

The following conclusions were drawn based on the outcomes of the present study:

1. Control group, induction of *H. pylori* without taking any plants or drug.
2. *Hepericum perforatum* group, prevented the stomach epithelium with two dosages (500,250 mg/ml) aqueous extract *Hepericum perforatum*, the results showed (50.65%) in high dose inhibition of ulcer area compared with control group, and (24.97%) in low dose inhibition of ulcer area.
3. *Punica granatum* group, the most effective plants that's used in the study was *P. granatum* high dose that (84.60%) inhibit ulcer area and low dose (42.87%) compared with control group ulcer area.
4. Omeprazole drug group, the least effective on gastric epithelium was omeprazole at rate (24.50%) inhibit ulcer area compared with control group.

6.2 Recommendation

1. Application of aqueous extract of *Punica granatum* as antimicrobial activity in protection gastric epithelium.
2. For future research, recommend studying the effects of *Punica granatum* on the physiology section.

3. Further works recommended performed finding the most effective doses for the antioxidant, ant-inflammation effect of *Punica granatum* peel extract.

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زانکۆی سەلاحەدین - هەولێر
کۆلێژی پەروەردە
بەشی بایۆلۆجی

NO: 140
Date: 11-4-2021

Approval of Research Ethical Committee

Herewith we declare that ethical committee / scientific researches office in research center of Salahadin University has processed the submitted research proposal of (M.Sc. Mohammed M.Hussein M.Raouf) master student from (Near East University). His research (**Effect of *Hypericum perforatum* & *Punica granatum* on Gastric ulcer in rat**) approved to be conducted in the setting of our research center.

Head of Biology Department
Asst.Prof.Dr. Kazhal M. Sulaiman
11/4/2021

Prof. Dr. Hikmat Ali
Post gruduate office