In Vivo Study of the Effects of Origanum Vulgare Aqueous Extract on Ethanol-Induced Gastric Ulcer in Sprague Dawley Rat

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Abstract— **Gastric ulcer, characterized by the erosion of the stomach mucosa, represents a persistent condition rooted in the delicate balance between aggressive factors and mucosal defenses. This study aimed to evaluate the comparative efficacy of herbal medicine derived from Origanum Vulgare against the conventional drug omeprazole in ulcer prevention. Utilizing Albino male rats, several experimental groups were formed: a positive control group induced with ethanol and dissected after one hour, a negative control group fed normally, rats induced with ethanol and pre-treated with Origanum Vulgare aqueous extracts at both low (300mg/kg) and high (600mg/kg) doses, and a group administered with the standard drug omeprazole at 20mg/kg. Results revealed notable ulcer inhibition percentages for Origanum Vulgare at both high (85.75%) and low (81.68%) doses, compared to omeprazole (87.38%). The observed ulcer inhibition rates in Origanum Vulgare groups were significantly superior to both the omeprazole and control groups. Additionally, Origanum Vulgare exhibited a substantial reduction in stomach index and cellular proliferation, accompanied by diminished cell damage. These findings underscore the heightened efficacy of higher doses of Origanum Vulgare aqueous extracts over lower doses in ulcer prevention.**

Index Terms— **Gastric Ulcer, Medicinal plant, Omeprazole, Origanum Vulgare, peptic ulcer**.

I. Introduction

Gastric ulcer in industrialized nations where there is discontinuity in the stomach mucosa, one of the most prevalent gastrointestinal issues and common illnesses occurs [1] [2]. Examples of ulcers in the digestive tract include open lesions on the stomach or eroding mucosal tissue layers [3] [4]. The term "gastric ulcer disease," also referred to as "peptic ulcer disease," denotes a perforation in the stomach's gut lining that has been open long enough to cause irritation of the mucosal barrier when exposed to sufficient amounts of the enzyme pepsin and acidic conditions. Various etiological factors, including stress, cigarette use, poor nutrition, infections, and frequent, indiscriminate NSAID use (NSAIDs), can result in stomach ulcers [5, 6]. The term "mucosal defense" describes a multitude of systems that maintain the mucosa's health even when exposed to harmful substances at various pH levels, osmolality, and temperatures [7] [8]. Additional potential triggers of local and systemic inflammation include cytotoxic or detergent-like chemicals, as well as different bacterial products [9] [10]. Omeprazole a medication used to treat or prevent a number of illnesses, including corrosive esophagitis, infection with H. pylori, pathologic hyper secretory disorders, ulcers of the stomach and duodenum, and is known as gastrointestinal (GI) reflux disorder [11] [12]. The generation of acidic is commonly reduced by the use of substances called proton pump inhibitors (PPIs) [13] [14]. For the management of bleeding from ulcers, these drugs are used as a quadruple or even triple treatment. The negative consequences of these drugs include the emergence of further chronic illnesses. These issues are avoided by using herbal remedies, which also provide a long-lasting solution [15] [16]. Origanum Vulgare the botanical family Origanum tends to be widespread via the globe's climatic regions but is most prevalent in the southern Mediterranean region [17] [18]. The substance found in the plant's extraction, which is abundant in phenolic antioxidants and has strong antibacterial and anti-fungal activities, is chiefly responsible for O. vulgare's positive advantages for health [19] [20].The rising threat that antibiotic resistance provides to global health has attracted scientists' attention to antimicrobial investigations throughout the previous twenty years [21] [22]. According to Chinese medicine, oregano is used to treat influenza, fever, nausea, diarrhea, yellowing of the skin and malnutrition in kids. [23] [24]. The field of traditional medicine apply this plant's extract as a sedative, hepatoprotective, antioxidant, anticancer, anti-diabetic, anti-inflammatory, antiviral, and fungicide [13] [25].

This study aims to compare the effectiveness of Origanum vulgare plant extracts and omeprazole in treating gastric ulcers, using male albino rats as the disease model.

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II. Material and Methods

A. Extract Preparation

In this scientific study, Origanum vulgare extract was administered to rats in two doses: a high dose of 600 mg/kg of powder extract in 6 ml of distilled water, and a low dose of 300 mg/kg of plant powder in 6 ml of distilled water. The preparation involved boiling the water until steam appeared, then allowing it to cool to 85°C before mixing it thoroughly with the powder. The mixture was then covered with aluminium foil and kept in a dark place at room temperature for 24 hours. After this period, the extracts were filtered using filter paper.

B. Omeprazole Preparation

Omeprazole, offered in varying strengths of 10, 20, and 40 mg through tablets, capsules, and intravenous solutions, undergoes swift degradation within the acidic milieu of the stomach. Its breakdown within the small intestine typically spans a duration of 3 to 6 hours. Consequently, omeprazole stands as the primary therapeutic choice for gastric ulcer patients globally. In the context of this research, a solitary omeprazole capsule containing 20 mg was dissolved in 7 cc of distilled water [26].

C. Experimental Animals

Healthy male Sprague Dawley rats weighted around 150 g to 180 g from the experimental animal, were collected from College of Science, Biomedical sciences Department, Cihan University-Erbil animal house, they were kept in several cages' normal humidity, lighting, and temperature conditions and were served with enough food and water.

The Ministry of Health's (MOH) Guidelines for the Care and Use of Laboratory Animals were followed when handling rats. To prevent a response between the previously treated medicines and the stomach contents during the experiment, all rats were allowed to fast for around 24-48 hours before to the experiment. To prevent dehydration.

D. Experiment procedure

Rats were divided into groups at random and given treatment in accordance with the specified guidelines. The treatments were given orally via the rats from their mouths by gavage and an amount of one millilitre. Following an hour administration of the therapy, the rats are provided pure ethanol to cause stomach ulceration. The rats were dissected one hour later with dosages of Xylazine and Ketamine. The scissors are used to perform a tiny cut across the rats' midline incisions. To stop the stomach contents from spilling out, the esophageal and pyloric ends were tied shut with strings. The stomach was then removed and placed in an ice-cold container.

E. Acute Toxicity

Following the administration of Origanum vulgare, the rats was monitored for 24-48 hours for any toxic symptoms. The typical interval between animal fatalities was believed to be more than two weeks. Rodents were injected with xylazine and ketamine on the fifteenth day observing that. Through an intracardial incision.

F. Gross Lesion Evaluation

The stomachs were scraped with a scalpel following being slowly rinsed under running water. The string at the esophageal end was removed. To determine the mass of the mucus, all of the contents of the stomach were squeezed into a small container. A lengthy, bleeding lesion parallel to the lengthy shaft of the stomach mucosa can be an ulcer. The pretreatment's anti-ulcerogenic action is greater when there is a higher inhibitor percentage and a smaller ulcer area.

G. Mucous Weight Measurement

The mucus and the other stomach contents were obtained after the stomach was opened, and every container was weighed using an electronic scale used.

H. Evaluation of Gastric PH

The pH of the gastric mucus in the rats' stomachs was evaluated using a pH meter to determine the intra-gastric acidity. This measurement assesses the acidity level of the mucus membrane, which forms a continuous network and traps bicarbonate released by the epithelium.

I. Histological Examination

After observing gross lesions, slice the open intestines into tiny pieces using the scalpel handle in the position across of the first incision across of lesions. Small portions of stomach were then placed into cassettes and stored in a 10% buffered formalin solution for the processing of tissue in order to maintain the stomach undamaged. Next, the cassettes were placed in 70% alcohol, followed by 95% alcohol, and finally absolute alcohol before being placed in xylene. Using a microtome, the tissues were sectioned, fixed in paraffin, and stained with H&E solution. The dyed tissues were then examined under a light microscope at magnifications of X10, X40, and X100 to detect mucosal layer damage in the tissue's structure.

III. RESULTS

A. Evaluation of the Impact of Origanum vulgare on Gastric Lesion Development

The formation of ulcers marked on by the full amount of ethanol has been examined in tremendous detail. Before and shortly after the incision of the stomach across the larger curvature, the development of ulcers was visually assessed. In comparison to the group previously treated with 5% CMC (ulcer control) The stomach mucosal underwent severe, apparent hemorrhage necrosis see figure 1. and the Negative control group show no lesion figure 2. 12 rats served as models in the in-vivo acute toxin testing. The rats were divided into two groups: the control group (G1) and the experimental group (G2). The control group received a dose of 600 mg/kg of Origanum vulgare, resulting in very mild macroscopic necrosis of the gastric mucosa. The experimental group received a dose of 300 mg/kg of Origanum vulgare, leading to mild to moderate macroscopic necrosis of the gastric mucosa (see Figures 3.3 and 3.4).

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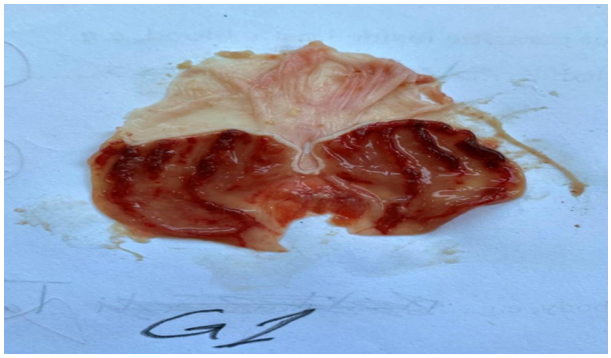

Fig. 3.1. Severe Macroscopic Necrosis of Gastric Mucosa

Fig. 3.2. No macroscopical lesion was found in Normal control

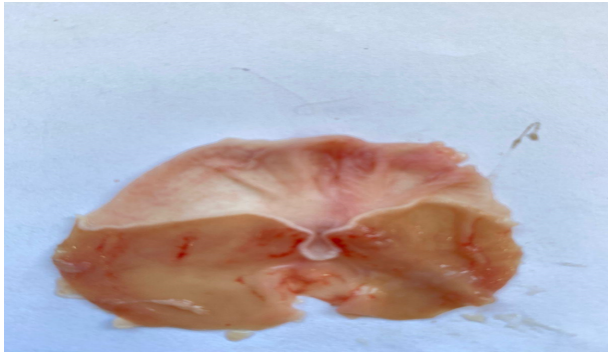

Fig. 3.3. Very mild macroscopic necrosis of gastric mucosa
Gastric mucosal damage caused by absolute ethanol in rats (pre-treated with 600mg/kg Origanum vulgare). Origanum vulgare reduces the development of gastric lesions caused by pure ethanol.

Fig. 3.4. Mild to moderate macroscopic necrosis of gastric mucosa.
Gastric mucosal damage caused by absolute ethanol in control animals (pretreated with 300mg/kg Low Dose Extract Origanum vulgare). reduces the formation of gastric lesion induced by absolute ethanol.

B. Histological evaluation of stomach

Histopathological investigation has proven that whether a low or a high dosage of Origanum vulgare administration can avoid ethanol-induced stomach mucosal necrosis, edema, hemorrhage, and ulceration.

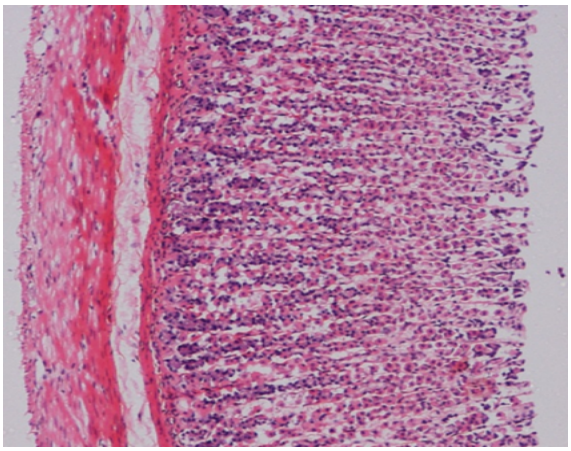

Fig. 3.5. Histological section of gastric mucosa (Normal control).

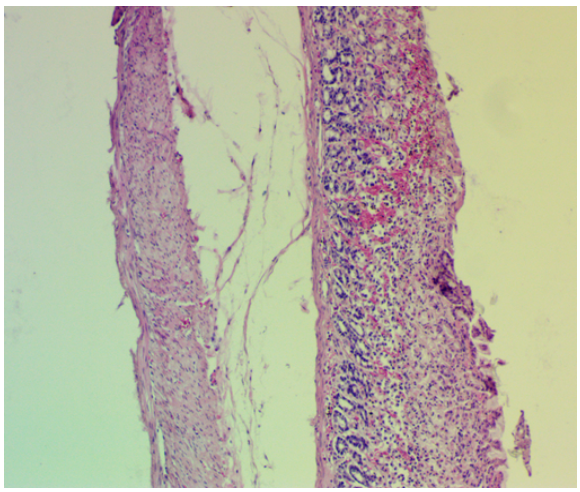

Fig. 3.6. Histological section of gastric mucosa in a rat pre-treated with 300 mg/kg of Origanum Vulgare.

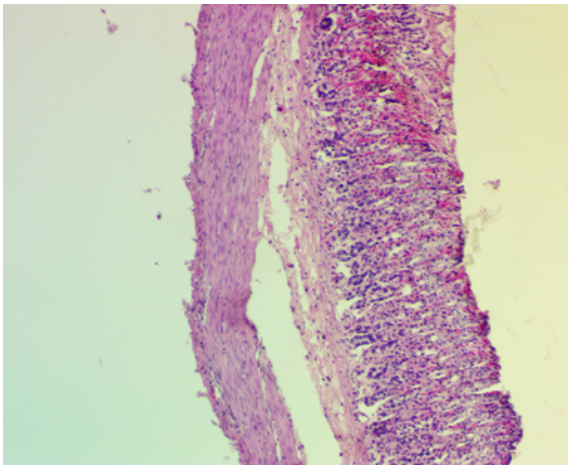

Fig. 3.7. Histological section of gastric mucosa in a rat pre-treated with 600 mg/kg of Origanum Vulgare.

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C. Histopathology evaluation of acute toxicity for liver and kidney

The in-vivo acute toxicity trial was conducted on 24 rats, which were divided into four groups. The experimental groups (G1, G2, G3, and G4), (G1and G2) received single doses of 300 mg/kg and (G3, G4) received 600 mg/kg of Origanum vulgare, respectively. The rats were observed every eight hours for 14 days. Throughout the observation period, the acute toxicity results indicated that all the experimental rats passed the test without any signs of toxicity. Moreover, follow-ups revealed no abnormalities in the rats' weight, behavior, food intake, or liquid consumption. Additionally, histopathological analysis showed no tissue damage in the liver and kidneys of the experimental rats.

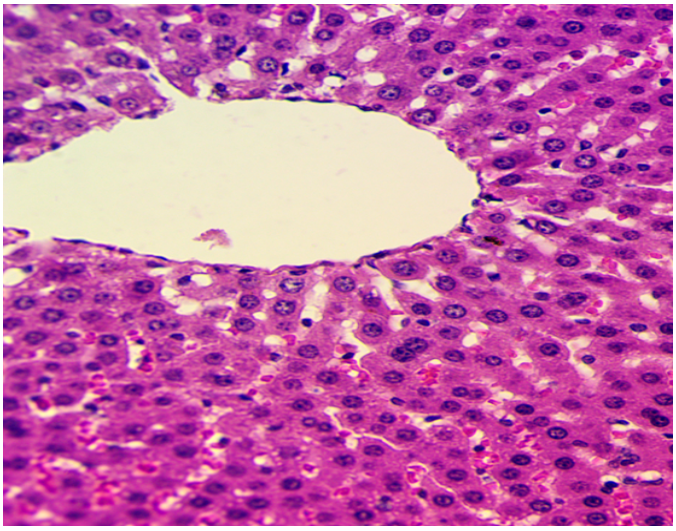

Fig. 3.8. Histological sections of the liver in the acute toxicity test (H&E staining, 40×). Rats were treated with 300 mg/kg (6 mL/kg). Origanum vulgare. No significant changes were observed in the structures of the liver between the treated and control groups (H&E staining, 40×).

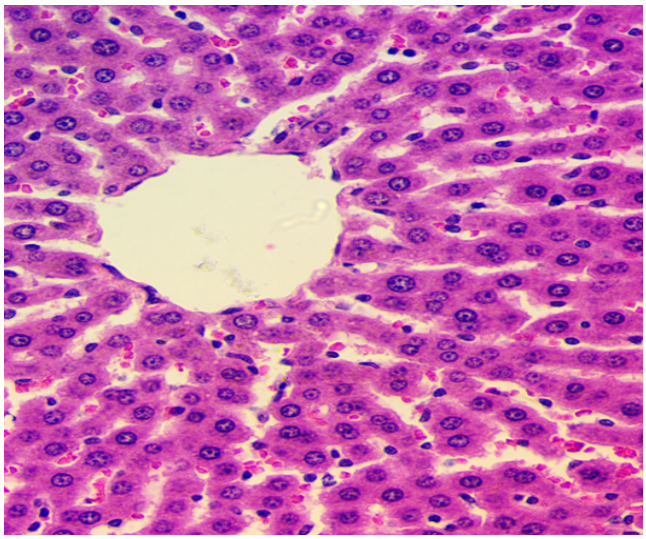

Fig. 3.9. Histological sections of the liver in the acute toxicity test (H&E staining, 40×). Rats were treated with 600 mg/kg (6 mL/kg). Origanum vulgare. No significant changes were observed in the structures of the liver between the treated and control groups (H&E staining, 40×).

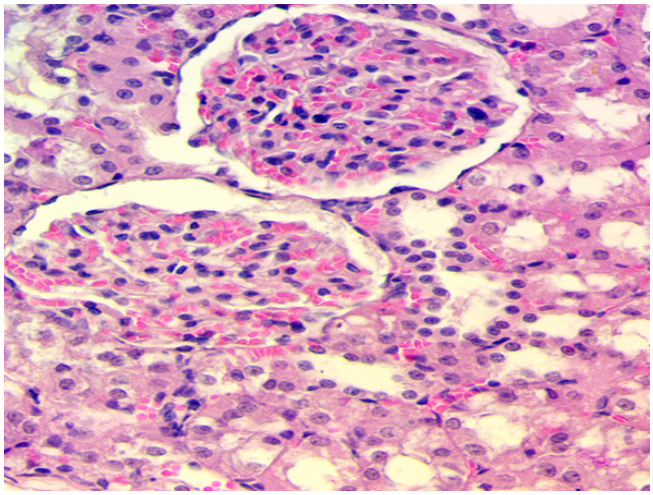

Fig. 3.10. The acute toxicity test shows histological sections of the kidney (H&E staining, 40×). Rats were rats treated with 300 mg/kg (6 mL/kg). Origanum vulgare. No notable differences were detected in the kidney structures between the treated and control groups (H&E staining, 40×).

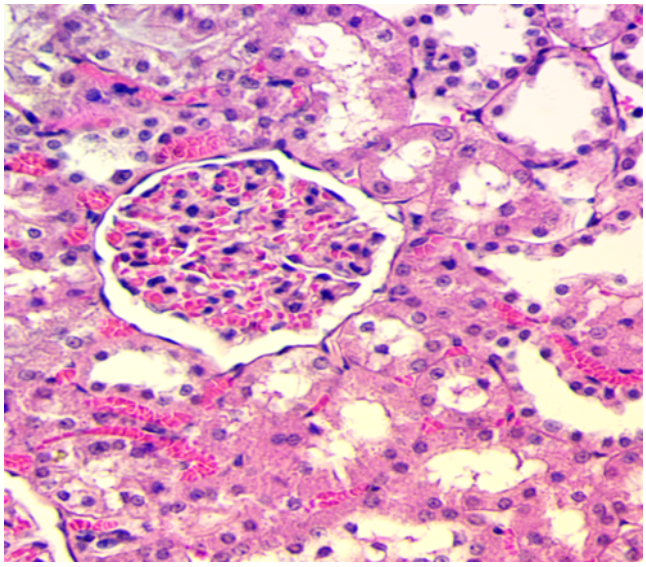

Fig. 3.11. The acute toxicity test shows histological sections of the kidney (H&E staining, 40×). Rats were rats treated with 600 g/kg (6 mL/kg) Origanum vulgare. No notable differences were detected in the kidney structures between the treated and control groups (H&E staining, 40×).

D. Statistical analysis

The statistical significance of differences between groups was assessed using ANOVA test. A probability value of $p < 0.05$ was considered to be statistically significant, ANOVA test = 0.0017 between Ph level of Control negative group, high dose 600 mg/kg Origanum vulgare, and low dose 300 mg/kg Origanum vulgare. ANOVA test = 0.0086 between mucus of control negative group, high dose 600 mg/kg Origanum vulgare and low dose 300 mg/kg Origanum vulgare. Based on Friedman test = 0.18 the result it was not significant between mucus of Omeprazole group, high dose 600 mg/kg Origanum vulgare and low dose 300 mg/kg Origanum vulgare.

P. value shown <0.005 significant between mucus of Omeprazole group, high dose 600 mg/kg Origanum vulgare and low dose 300 mg/kg Origanum vulgare.

P. value shown < 0.0001 between Omeprazole group, high

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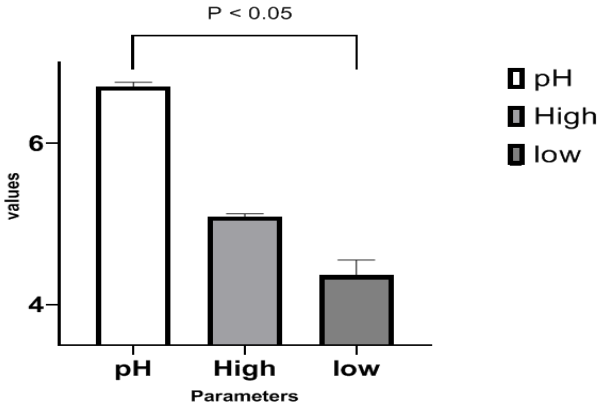
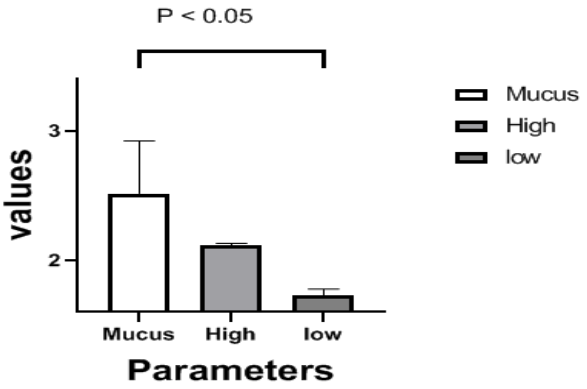
dose 600 mg/kg Origanum vulgare and low dose 300 mg/kg Origanum vulgare.

Inhibition percentage P. value shown < 0.0001 between Omeprazole group, high dose 600 mg/kg Origanum vulgare and low dose 300 mg/kg Origanum vulgare.





IV. Discussion

The regular balance among the protective systems of mucus, bicarbonate, mucosal employee turnover, and circulation and the damaging elements of acid pepsin is disrupted in a condition called peptic ulcer or stomach ulcer disease. regular balance among the protective systems of mucus, bicarbonate, mucosal employee turnover, and circulation and the damaging elements of acid pepsin is disrupted in a condition called peptic ulcer or stomach ulcer disease [27]. Considering the fact that the etiology of ulcers remains frequently unclear [28]. it is commonly accepted that these conditions result from an imbalanced interplay among external forces and the capacity of the endogenous defense system to maintain the arrangement of the mucosa [28]. It was additionally discovered that hemorrhaging drugs like ethanol decrease these stomach defense systems when gastric lesions of the mucous membrane build up [29] [30].

The purpose of the present research aimed to evaluate the anti-ulcer properties of Origanum vulgare extracts towards Alcohol damaged rats' stomachs. Omeprazole has been considered in this study as a comparative drug because it is frequently used for the treatment of ulcers in the stomach. Omeprazole, one of the protons pumps inhibiting agents, is employed for the treatment of peptic ulcers, Zollinger-Ellison syndrome (LES), laryngopharyngeal reflux, dyspepsia, and reflux of the oesophagus. This discovery confirms Omeprazole's ability to boost stomach secretion of mucus [31]. In addition to the effects of intra-gastric acidity, also. By impacts on the proton pump, omeprazole inhibits the final stage of acid formation, hence reducing intra-gastric acidity. It examined whole ethanol management to induce serious ulceration in the gut lining in rats. Since total ethanol may easily enter the gastric mucosa in a brief period of time [32] [33]. this method is appropriate for examining an anti-ulcerogenic effect [34].

Results of the present investigation further demonstrate that orally administered Origanum vulgare strongly protected the gastric mucosa against ulcer production by 100% ethanol and inhibits leukocyte invasion at the sub-mucosal layer in rats, The result agrees with the finding of this research [35] [36]. which employed two doses of Origanum vulgare extract, indicate that a low dosage is more efficient than a high dose in avoiding stomach ulcers. These findings are consistent with those of [37] This study showed that Origanum vulgare plant extracts were tested for acute toxicity at doses of 300 mg/kg and 600 mg/kg, the results proved no evidence of toxicology or death rates [38]. There were also not significant variations in the overall structure of the liver between the treated and control groups. No discernible differences in the kidney's structure between treatment and control groups were discovered for extracts in those areas if experimental animals' liver and kidneys remained unaltered throughout this investigation.

Conclusion

The extracts of Origanum vulgare proved an anti-ulcerogenic effect on rats with artificially produced ethanol-induced ulcers in the stomachs. Test animals can avoid getting ulcers brought on by ethanol when given (Origanum Vulgare) at dosages of 300 mg/kg (low dosage) and 600 mg/Kg (high dose). The stomach mucosa is likewise defended by the extra mucous resistance as a response to the externally hostile element. Origanum Vulgare appears to have gastroprotective effects based on increased mucus production and an increase in stomach acidity pH values. Furthermore, the histopathology demonstrated that Origanum vulgare at a high dose 600 mg/Kg was more significant to a low dose 300 mg/Kg, and high dose 600 mg/Kg protect the stomach mucosa and tumors and leucocyte invasion intramuscularly.

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