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Histological and Histochemical Study of the Effect of the Alcoholic Extracts of licorice and Purslane, in Combination with Omeprazole, on Experimentally Induced Gastric Ulcers in Albino Rats

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Abstract This study was conducted in the animal house of the Department of Life Sciences / College of Education / Al-Qadisiyah University. The current study aimed to evaluate the role of the alcoholic extract of the plants Licorice and Purslane compared with the drug Omeprazole in treating gastric ulcers induced by aspirin through physiological study during the period between 2/3/2025 and 20/4/2025. (50) male albino rats, weighing between (180-200) grams and aged between (12-14) weeks, were used. They were divided into a negative control group (C) comprising (20) rats, which were given the standard diet and distilled water only, and treatment groups (T) comprising (30) rats in which gastric ulcers were induced by administering (100) mg/kg of body weight of aspirin orally for (7 and 14) days. After the end of the experimental period of (50) days, the animals were anesthetized with chloroform and left for approximately three minutes, then the stomachs were removed and preserved in 10% formalin for histological study. Histological examination using hematoxylin staining revealed pathological histological changes in treatment group (T1), characterized by hemorrhages in the epithelial cells of the gastric mucosa. Mild congestion was also observed in the submucosal layer (MTS) with infiltration of inflammatory cells. In treatment groups (T2), (T3), and (T4), histological sections showed a marked improvement in the gastric wall layers compared to treatment group (T1). Histological sections using PAS (Periodic Acid-Schiff) and Masson's Trichrome Staining revealed a thin layer of mucus and colloidal fibers in treatment group (T1), while a gradual increase in gastric tissue was observed in treatment groups (T2), (T3), and (T4).

Keywords: Licorice, Purslane, Omeprazole, Gastric Ulcer, White Rats

Introduction Gastric ulcers are a common digestive disorder affecting about 10% of the global population (1). They result from an imbalance between aggressive factors, such as gastric acid and pepsin, and protective factors like mucus and bicarbonate, leading to damage of the gastric mucosa (2). The primary causes include the use of non-steroidal anti-inflammatory drugs (NSAIDs), especially aspirin, and infection with Helicobacter pylori, both of which weaken mucosal defenses and increase acid secretion (3). Although conventional treatments such as proton pump inhibitors (e.g., omeprazole) and H2-receptor antagonists are effective, they may produce adverse effects in some patients (4). Therefore, attention has increasingly shifted toward natural therapies with fewer side effects. Medicinal plants have long been used in traditional medicine and are gaining scientific interest due to their bioactive compounds, safety, accessibility, and cost-effectiveness (5,6). Licorice (Glycyrrhiza glabra) is a widely used medicinal plant rich in active compounds, particularly glycyrrhizin, with various

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pharmacological activities including anti-inflammatory, antiviral, and anti-ulcer effects (7–12). It has been used for centuries in both Eastern and Western medicine and is also approved as safe by the U.S. Food and Drug Administration for certain uses (13). However, excessive consumption may cause side effects such as hypertension and electrolyte imbalance (14).

Another important medicinal plant is purslane (Portulaca oleracea L.), which has been classified by the World Health Organization as a widely used medicinal plant (15). It is rich in bioactive compounds, including flavonoids, vitamins, and minerals, and exhibits antioxidant, antimicrobial, antidiabetic, and anti-ulcer properties. Its nutritional and therapeutic value has attracted increasing interest in both research and industry (16) Omeprazole, a proton pump inhibitor discovered in the late 1970s, acts by inhibiting gastric acid secretion through blocking the H^+/K^+-ATPase enzyme in parietal cells (17). It is widely used in the treatment of gastric ulcers and related disorders, although long-term use may be associated with adverse effects (18). This study aims to evaluate the effect of alcoholic extracts of licorice and purslane on experimentally induced gastric ulcers in albino rats, in comparison with omeprazole, using histochemical techniques including PAS, Masson's trichrome, and H&E staining.

Materials and Methods

Ethical approval

The project was approved (14623 in 3/3/2026) by the Committee for Research Ethics at the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

Plant Sample Preparation

Licorice-Glycyrrhiza (Licorice) plants (Purslane - Portulace oleracea) and Purslane glabra were obtained from local markets in Diwaniyah. They were ground using an electric grinder to obtain the powder, which was then stored in glass containers until extraction.

Preparation of the Alcoholic Extract of Licorice Root.

The plant roots were ground using a grinder, and then the ground parts were sieved through sieves with holes of (2 mm). The powder was collected and left at a temperature of (45 m) for 72 hours. 20 grams of the dry powder were weighed and placed in a soxolite apparatus to start the extraction process using (400 ml) of 99.9% absolute ethanol as a solvent to obtain the alcoholic extract. The dry powder to solvent ratio was thus 20:1. The extraction process continued for 24 hours per batch. The resulting extract was then placed in clean, sterile glass dishes and incubated at 45°C for 24 hours to dry. After weighing, the extract was stored in clean, airtight plastic containers until use. (19)

-Preparation of Purslane Alcoholic Extract

The plant was ground using a grinder, and the ground material was then sifted through sieves with a 2 mm mesh size. The powder was collected and left at 45°C for 72 hours. Twenty grams of the dry powder were weighed and placed in a soxolizer to begin the extraction process using 400 ml of 99.9% absolute ethanol as the solvent. The dry powder to solvent ratio was 20:1. The extraction process continued for 24 hours per batch. The resulting extract was then placed in clean, sterile glass dishes and incubated at 45°C for 24 hours to dry. The extract was then weighed and stored in clean, airtight plastic containers until use. (19)

Preparation of Laboratory Animals

The experiment was conducted in the Animal House/Department of Life Sciences, College of Education, Al-Qadisiyah University, from March 2, 2025, to April 20, 2025, using (50) male albino rats aged (12-14) weeks, weighing between (180-200) grams. They were placed in a climate-controlled room with a suitable temperature between (22-25) °C and a lighting system of (12) hours of light - 12 hours of darkness. The animals were provided with water and a standard diet throughout the experiment and were left for two weeks to acclimatize and adapt. They were then divided as follows:

First - The control group (C): its number is (20) rats.This was considered the negative control group and this group was given only their usual diet and distilled water for the entire 30-day duration of the experiment.

Second - Treatment group (T): This consists of four groups totaling (30) rats in which ulcers were induced by administering aspirin (100 mg/kg of

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body weight) for (7, 14) consecutive days. After confirming the induction of ulcers, they were divided as follows:

A.The first treatment group (T1): It included (6) animals in which gastric ulcers were induced and considered a positive control.

B.The second treatment group (T2): included (6) animals in which gastric ulcers were induced and they were orally administered omeprazole at a dose of 20 mg/kg of body weight for 30 days.

C.Treatment group three (T3): included (6) animals in which gastric ulcers were induced and which were orally administered the alcoholic extract of purslane at a dose of 100 mg/kg of body weight for 30 days.

D.Treatment group four (T4): This group included (6) animals in which gastric ulcers were induced and which were orally administered an alcoholic extract of licorice root at a dose of 100 mg/kg of body weight for 30 days.

Aspirin Solution

Aspirin powder was prepared from a stockpile to obtain the required dose of 100 mg/kg body weight by dissolving (1) molecule of aspirin powder in (100) ml of 1% carboxymethyl cellulose. The solution was then administered at a dosage of 1 ml per 100 g/animal weight. (20)

Preparation of Omeprazole

Omeprazole was used to treat aspirin-induced gastric ulcers in animals by preparing a solution with a concentration of 20 mg/kg body weight. This was achieved by dissolving 0.2 g of omeprazole powder in 100 ml of distilled water to obtain a concentration of 2 mg/ml. Each rat received 1 ml per 100 g of body weight. (21)

Induction of Gastric Ulcers

Gastric ulcers were induced in all experimental groups except the negative control group by administering aspirin (100 mg/kg body weight once daily after a 14-hour fast) before given aspirin for 714 consecutive days. Three animals from each group were then sacrificed to confirm ulcer formation. Aspirin administration was continued for a second week until ulcer development was confirmed (22)

As for the preparation of tissue slides, they were prepared according to the method of (23). Hyatoxylin-eosin stain was used to stain the samples following the steps described by (24). Massones Trichrome stain was used to stain colloidal fibers, and periodic acid-Schiff stain (PAS) was used to stain the carbohydrate material (mucus) according to the method of (25).

Microscopic Examination and Imaging of Samples

A light microscope was used to examine tissue slides to identify the studied histological changes. The tissue sections were imaged using a digital camera connected to the microscope at an appropriate magnification.

Statistical Analysis

The experimental data were statistically analyzed using one-way ANOVA (Analysis of Variance) in the SPSS statistical software to determine the differences between the means of the experimental groups. Significant differences were selected using the least significant difference (LSD) at the probability level criterion ($p > 0.05$) (26)

Histological Study of the Stomach:

Hematoxylin-Eosin

The results of the microscopic examination of the tissue sections taken from the stomach of the negative control group (C) fed on the normal diet and freely distilled water shown in Figure (1) and stained with hematoxylin-eosin stain the normal histological structure of the stomach wall showed that it consists of four main layers, arranged from the inside out: the mucosa layer, in which the epithelial tissue is modified into gastric pits with several gastric glands opening at their bases; below these glands lies the muscular mucosa layer, which rests on loose connective tissue representing the submucosa layer, bordered externally by the muscularis externalis layer.

In contrast, the microscopic examination of the stomach sections from the rats in the treatment group (T1) showed pathological histological changes, specifically hemorrhages in the cells. The epithelium of the mucous layer of the stomach wall, as well as the presence of slight congestion in the submucosa layer with the presence of infiltration of inflammatory cells in the submucosa layer as shown in Figure (1).

While microscopic examination of tissue sections treated with omeprazole (20 mg/kg) (2) showed that the normal gastric histological

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structure consisted of four main layers, with mild congestionas illustrated in Figure (2).

On the other hand, the results of the microscopic examination of the tissue sections taken from the stomach of the group treated with purslane (100 mg/kg) (T3) showed the normal stomach tissue, the mucous layer and gastric glands. The results of the microscopic examination of the tissue sections taken from the stomach of the group treated with licorice root (100 mg/kg) (T4) also showed the normal stomach tissue, the mucous layer and gastric glands. We also note the muscular layer as shown in Figure (3).

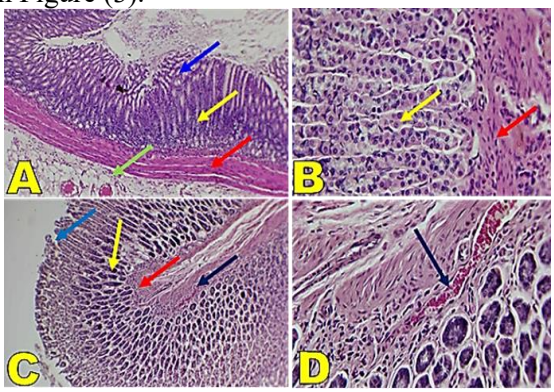

Figure (1) shows a cross-section of gastric tissue. Images (A&B) represent the control group. The gastric tissue appears normal: serous layer (green arrow), muscular layer (red arrow), submucosa (yellow arrow), and mucosa (yellow arrow). Images (C&D) represent the treatment group (T1). Hemorrhage is observed in the epithelial cells (green arrow) of the gastric mucosa, along with infiltration of inflammatory cells (black arrow). (H&E, 10X, 40X)

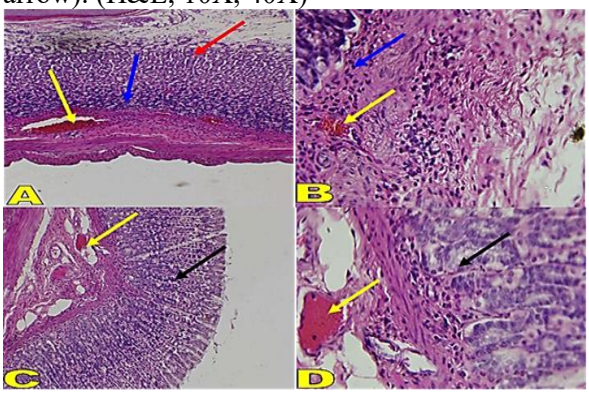

Figure (2) shows a cross-section of gastric tissue. Images (A&B) indicate the treatment group (T1). We observe congestion in the submucosa (yellow arrow) and infiltration of inflammatory cells in the submucosa (blue arrow). Images (C&D) indicate the treatment group (T2). We observe mild congestion (yellow arrow) and normal gastric tissue (black arrow). (H&E, 10X, 40X)

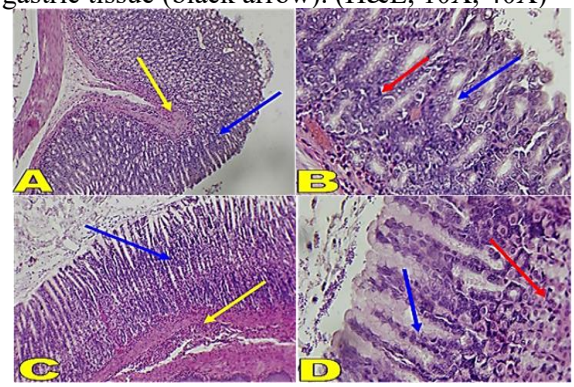

Figure (3) shows a cross-section of stomach tissue. Images (A&B) indicate the treatment group (T3). We observe the presence of normal stomach tissue, including the mucous layer and gastric.

glands (blue arrow), as well as the muscular layer (yellow arrow). Image (CD) shows the treatment group (T4). We observe the presence of normal stomach tissue, including the mucous layer and gastric glands (blue arrow), as well as the muscular layer (yellow arrow).

Histochemical analysis:

PAS stain:

Histological examination using PAS staining for mucosal detection in the negative control group C revealed a normal gastric histological structure. All layers of the gastric wall were observed, showing strong mucosal cell reaction in the gastric fossa.

Microscopic examination of the positive control group (T1), treated with aspirin only (100 mg/kg), showed a marked decrease in mucosal reaction to PAS staining, indicating a significant reduction in mucosal secretion. No positive reaction was observed in the gastric fossa cells compared to the negative control group, indicating clear damage to the gastric mucosa.

Histological sections of the T1 group treated with the ulcer-inducing agent showed clear pathological changes, including hemorrhage in the mucosal epithelial cells, congestion in the submucosa, infiltration of inflammatory cells, and

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marked destruction of the mucosa, along with the formation of ulcerative lesions, a significant decrease in mucin secretion, and the absence of a positive PAS stain in the gastric pits.

In contrast, the results of the omeprazole treatment group (T2) at a dose of 20 mg/kg showed significant improvement. The mucosa exhibited a strong reaction to PAS staining, indicating increased mucus secretion. Epithelial cells in the gastric fossa showed a moderate reaction to the staining compared to the control group. Additionally, small amounts of free PAS-positive mucin were observed on the mucosa surface, reflecting improved secretory function and mucosal protection.

The group treated with purslane extract (T3) at a dose of 100 mg/kg showed partial improvement, with moderate mucosal reaction to the dye, indicating a modest increase in mucus secretion. However, the epithelial cells in the pit area showed a weak reaction, with small amounts of free PAS-positive mucin present on the mucosal surface.

While the group treated with licorice extract (T4) at a dose of 100 mg/kg showed relatively better results, the mucosa exhibited a strong reaction with PAS staining, indicating a marked increase in mucus secretion. A moderate reaction was also observed in the cells lining the gastric fossa, with limited amounts of free PAS-positive mucin on the mucosa surface, suggesting a significant improvement in mucosal integrity and its protective function.

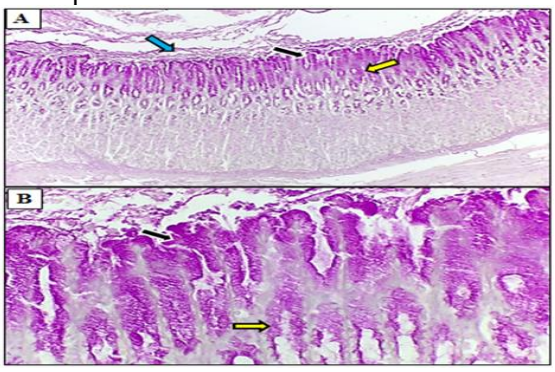

Figure 4: Cross-section of gastric tissue where image (A & B) indicates the control group T1. We observe strong PAS-positive reactions of neutral mucin in the cytoplasm of epithelial cells in the superficial mucosa (black arrow), extending to include the gastric pits (yellow arrow). PAS-positive free mucin was also observed. (A: 100x and B: 400x, blue arrow) above the surface of the gastric mucosa.

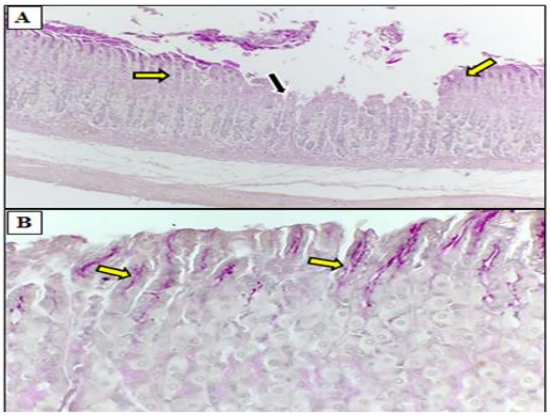

Figure 5: Cross-section of gastric tissue. Figures A and B indicate group T1, where severe destruction of the gastric mucosa led to the formation of an ulcerative lesion involving the superficial mucosa and the gastric pits (black arrow). Epithelial cells adjacent to the ulcerated area showed very weak reaction to neutral mucin PAS staining, with its presence limited to the cytoplasm of superficial epithelial cells (yellow arrow). No positive reaction to PAS staining was observed in the gastric pits compared to the control group. PSA, A: 100x and B: 400x

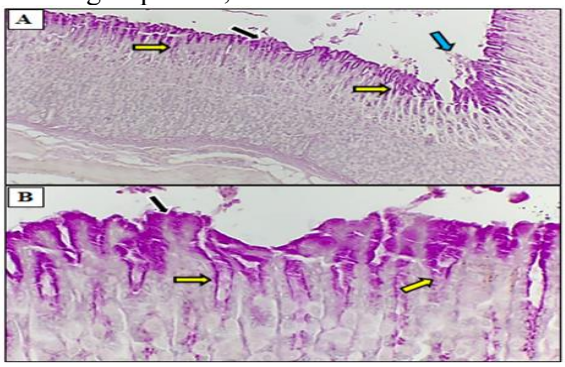

Figure 6: Cross-section of gastric tissue. Figures A and B indicate group 2, where the epithelial cells in the superficial mucosa (black arrow) showed a strong reaction to neutral mucin PAS staining, while the epithelial cells in the gastric pits (yellow arrow) showed a moderate reaction to PAS staining compared to the control group. Additionally, small amounts of free PAS-positive mucin were observed (blue arrow). A: 100x and B: 400x PAS staining.

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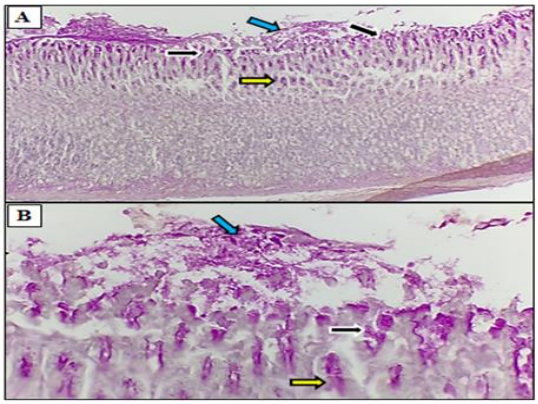

Figure (7) shows a cross-section of gastric tissue where images (A & B) indicate the T3 group. Moderate PAS staining of neutral mucin was observed in the epithelial cells of the superficial mucosa (black arrow), while weak staining was observed in the epithelial cells lining the gastric pits (yellow arrow) compared to the control and T2 groups. Additionally, small amounts of free PAS-positive mucin (blue arrow) were found on the surface of the PAS layer. A: 100x and B: 400x

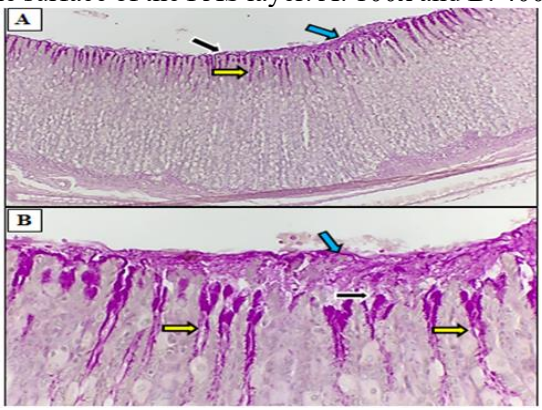

Figure 8: Cross-section of gastric tissue. Figures A and B indicate the T4 group. A strong reaction of neutral mucin to PAS staining was observed in the epithelial cells of the superficial mucosa (black arrow), while moderate staining was observed in the cells lining the gastric pits (yellow arrow) compared to the control group. Limited amounts of free PAS-positive mucin (blue arrow) were also found above the mucosa surface. PAS: A 100x and B 400x

Mason's Trichrome

The results of microscopic examination of the stomach sections taken from the negative control group (C) fed on the normal diet and free distilled water shown in Figure 9 and stained with Mason's trichrome stain showed the normal histological structure of the stomach. The normal histological structure of the stomach wall was shown, with collagen fibers clearly present in the damaged areas. The connective tissue forms between the neck, mid, and fossa regions and extends from the lamina propria. The collagen fibers showed a strong reaction with the stain.

This stain is a vital tool for assessing the integrity of the extracellular matrix and the extent of tissue fibrosis, as it stains collagen fibers blue. Microscopic examination of the control group (Figure 9) showed a regular distribution of fine collagen fibers that form the supporting connective tissue between the gastric glands, with clear visibility of the submucosa and the normal structure of the gastric wall. The four histological layers were clearly visible: mucosa, submucosa, muscularis, and serosa. In addition, the gastric glands and gastric pits were intact, and there was a regular distribution of collagen fibers in the lamina propria and submucosa.

While we observed in the microscopic examination of tissue sections taken from the treatment group (T1), a decrease in collagen fibers was noted in the damaged areas, as the collagen fibers showed a marked reaction with their specific stain, as illustrated in Figure 10.

While the T2 treatment showed preservation of the superficial layer of the gastric mucosa, the supporting basement membrane remained intact, and mild fibrosis appeared in the affected area as thin strands of connective tissue within the mucosa. Overall, the structural composition of the mucosa remained relatively preserved despite this mild fibrosis, as illustrated in Figure 11.On the other hand, microscopic examination of histological sections taken from the stomach of the group treated with purslane (100 mg/kg) (T3) revealed destruction of the superficial layer of the gastric mucosa, with dispersion of collagen fibers in the basement membrane, indicating severe structural damage, as illustrated in Figure (12).

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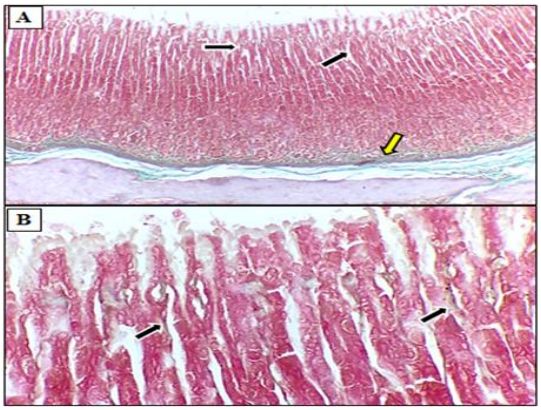

Figure 9: Cross-section of gastric tissue. Figures A and B represent the control group. The gastric mucosa in this group showed normal histological organization. Thin collagen fibers (black arrow) were observed forming the connective tissue between the neck and midline regions, the pits, and extending from the lamina propria. The submucosa (yellow arrow), composed of collagen fibers, was also clearly visible. All collagen fibers showed a positive Masson's Trichrome stain reaction. A: 100x and B: 400x

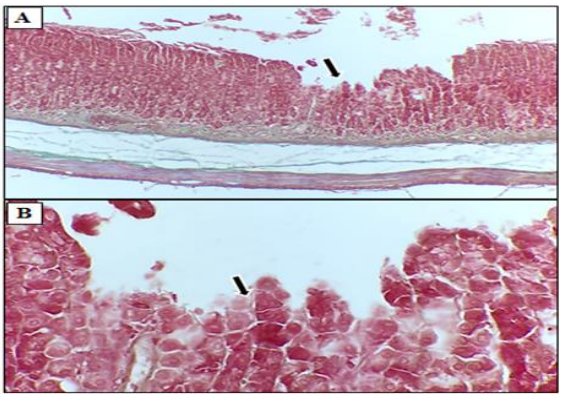

Figure (10) Cross-section of gastric tissue where image (A&B) indicates group 1. In the damaged areas of the gastric mucosa, collagen fibers were largely lost, with very few fibers remaining in the healthy areas beneath. No significant staining of the collagen fibers with Masson's Trichrome Stain was observed. A: 100x and B: 0.400x

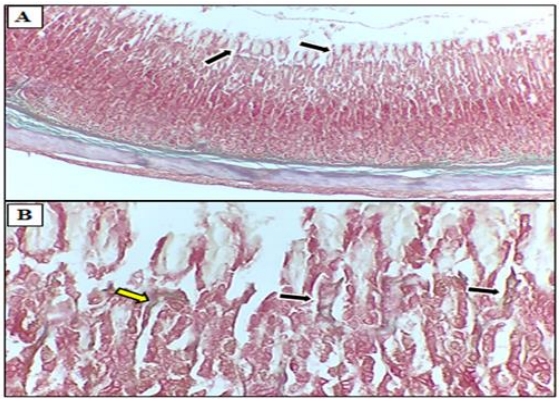

Figure 11: Cross-section of gastric tissue. Figures A and B show the T2 group. The superficial layer of the gastric mucosa was preserved, with the supporting basement membrane remaining intact (black arrow). Mild fibrosis (yellow arrow) was also present in the affected area, appearing as thin strands of connective tissue within the mucosa. Overall, the structural composition of the mucosa remained relatively preserved despite this mild fibrosis. Masson's Trichrome Stain. A: 100x and B: 400x.

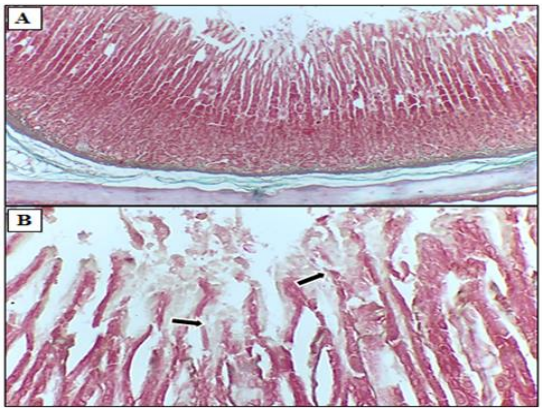

Figure 12: Cross-section of gastric tissue. Figures A and B show that the T3 group shows destruction of the uperficial layer of the gastric mucosa, with disorganized and scattered collagen fibers in the basement membrane (black arrow). Normal mucosal structure is also lost in the affected areas, indicating severe structural damage. (Massons Trichrome Stain, A: 100x and B: 400x)

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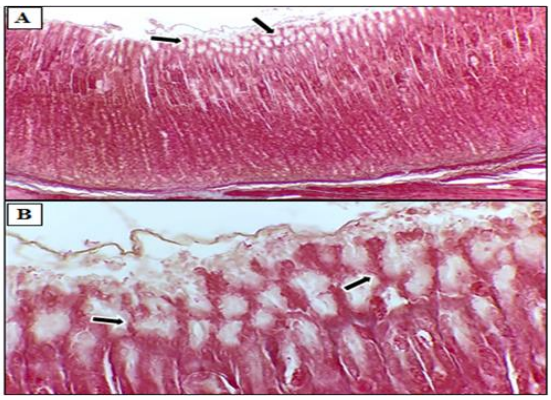

Figure 13: Cross-section of gastric tissue. Images A and B indicate group T4. Preservation of the superficial layer of the gastric mucosa was observed, with the supporting basement membrane remaining intact and organized (black arrow). Although the surrounding tissues underwent some changes, this superficial layer maintained its overall structural composition and mucosal surface continuity. Masson's Trichrome Stain. A: 100x and B: 400x

Discussion:

Histological Study of the Stomach:

Hematoxylin-Eosin:

In contrast, the microscopic examination of the stomach sections from the rats in the treatment group (T1) showed pathological histological changes, specifically hemorrhages in the cells. The epithelium of the mucous layer of the stomach wall.

These results are consistent with those found by (27) in their study of aspirin-induced ulcers, where they confirmed that irritating agents lead to loss of epithelial cell continuity and subepithelial hemorrhage. Park et al., (28) further support this finding, indicating that inflammatory stimuli cause impaired microvascular permeability, leading to the inflammatory infiltration observed in Figure (1.) While microscopic examination of tissue sections treated with omeprazole (20 mg/kg) showed that the normal gastric histological structure consisted of four main layers, this improvement is explained by the fact that omeprazole, as a proton pump inhibitor, provides a low-acid environment that prevents further tissue damage. This is supported by the study by Shinnawy et al. (27), who demonstrated that omeprazole significantly reduces the ulcer index and reorganizes the histological architecture by reducing inflammatory mediators, which is reflected in the reduction of congestion shown in Figure (2).

On the other hand, the results of the microscopic examination of the tissue sections taken from the stomach of the group treated with purslane (100 mg/kg) (T3) showed the normal stomach tissue, the mucous layer and gastric glands. The study by Uddin et al. (29) attributes this protection in the purslane group (T3) to its rich content of omega-3 fatty acids and antioxidants that act as cell repairers. These results are also consistent with those of Park *et al.* (28) and Aboulthana *et al.* (30), who found that plant extracts rich in phenols possess gastroprotective properties that prevent tissue breakdown and are comparable in efficacy to chemical drugs, which explains the healthy tissue appearance in Figure(3). Compared to the results of the current study, the severity of bleeding and inflammatory infiltration in (T1) was evident, indicating that the ulcer model used is effective in inducing acute tissue damage, which is consistent with the results of (39), who recorded similar changes using chemical ulcer models.

These findings are consistent with those of (27), who indicated that irritants such as aspirin lead to loss of epithelial cell continuity and subepithelial hemorrhage. Park *et al.* (28) further support this interpretation by demonstrating that inflammation increases microvascular permeability, thus explaining the observed inflammatory infiltration. Therefore, the current findings do not represent mere morphological changes, but rather reflect a comprehensive pathological mechanism involving oxidative stress and an inflammatory response.

Both purslane (T3) and licorice (T4) groups showed a tissue appearance close to normal, but this improvement cannot be explained simply as a "return to normal," but rather reflects the effectiveness of plant compounds in enhancing cellular protection mechanisms.

This result in the purslane group is attributed to its rich content of antioxidants and omega-3 fatty acids, which reduce oxidative stress and protect epithelial cells, consistent with Uddin et al. (29) While the effect of licorice is more

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pronounced due to its content of active compounds that stimulate anti-inflammatory mechanisms and enhance mucosal stability.

In the purslane group (T3), the results showed histological improvement with some signs of damage remaining, indicating a moderate protective effect. This is explained by the ability of the phenolic compounds and antioxidants in purslane to reduce oxidative stress, though not enough to completely halt deep structural damage. This is consistent with the findings of Alam *et al*. (32), who showed that purslane improves inflammatory markers but does not completely prevent severe histological changes.

While the licorice group (T4) showed near-complete restoration of tissue structure, indicating a strong protective effect, this is attributed to its flavonoid and glycyrrhizin content, which inhibit inflammation and promote epithelial cell regeneration. This finding is consistent with the 2020 study by Wang *et al*.,(20) which confirmed that licorice promotes gastric mucosal healing by inhibiting NF-κB inflammatory pathways. Comparing the groups reveals that tissue damage in (T1) is accompanied by gradual improvement in (T2), then (T3), and finally (T4), reflecting the different mechanisms of action between the treatments, ranging from pharmacological acid inhibition to cellular protection (plant-based).

Histochemical analysis:

PAS stain:

Microscopic examination of the positive control group (T1), treated with aspirin only (100 mg/kg), showed a marked decrease in mucosal reaction to PAS staining, indicating a significant reduction in mucosal secretion. No positive reaction was observed in the gastric fossa cells compared to the negative control group, indicating clear damage to the gastric mucosa. Figure (4) shows the strong and continuous PAS reaction in the surface cells and pits of the stomach, representing the protective mucosal barrier. This distribution is the first line of defense, as discussed in the study by Park *et al*. (28), where normal mucin content is an indicator of mucosal integrity and its ability to resist attacking agents. The examination shown in Figure (5) revealed acute mucin depletion (very weak reaction) with the appearance of a deep ulcer vacuole (black arrow). This is consistent with the findings of Al Batran et al. (33), who indicated that irritants (such as ethanol or aspirin) cause acute depletion of mucin in the gastric wall (GWM). El-Shinnawy et al. (27) confirm that this depletion is the precursor stage to tissue erosion and the formation of an apparent ulcer.

In contrast, the results of the omeprazole treatment group (T2) at a dose of 20 mg/kg showed significant improvement. The mucosa exhibited a strong reaction to PAS staining, indicating increased mucus secretion.

The results observed in Figure 6 reveal a strong mucin reaction in the surface layer and a moderate reaction in the gastric pits. This is consistent with Shin and Sachs (34) demonstrated: that protecting mucin-secreting cells from acid erosion allows them to maintain their secretory activity. The study by El-Shinnawy et al. (27) confirms that omeprazole increases the concentration of mucus bound to the cell wall, thus enhancing the positive response to PAS staining.

Histological sections of group T2 (Figure 6) showed a marked improvement in the gastric tissue structure, with relative integrity of the gastric mucosa observed with only slight congestion, in addition to the return of a positive PAS stain reaction in the surface epithelial cells, indicating the restoration of mucin secretion.

Group T3 in Figure (7) showed moderate myosin reactivity in surface cells and weak reactivity in gastric foci. Although the protection was less than that provided by omeprazole, the studies by Uddin et al. (29) and Aboulthana et al. (30) confirm that purslane compounds enhance cell membrane stability, preventing the complete loss of myosin observed in the infected group and maintaining a minimum level of biochemical protection

The results in Figure 8 show a strong myosin reaction in the surface layer and a moderate reaction in the pits, surpassing that of the T3 purslane group. This strong reaction aligns with the findings of Park et al. (28) regarding the ability of certain plant extracts to stimulate the

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production of prostaglandins (PG), which are the primary stimulators of myosin secretion. The results indicate that licorice possesses a potent stimulatory mechanism that restores myosin content levels to near normal.

These results were confirmed by the study of (35) which showed that the flavonoid compounds found in licorice promote the regeneration of mucous cells and increase mucin secretion by activating the EGFR/ERK pathway in the epithelial cells of the stomach. Also, the study by Panji and Ghafouri (36) showed that licorice compounds possess anti-inflammatory and antioxidant effects and contribute to reducing tissue damage in the stomach. Furthermore, a study by Guo et al. (37) demonstrated that licorice flavonoids can reduce gastric mucosal damage by inhibiting apoptosis and regulating the PI3K/AKT pathway, thereby accelerating gastric ulcer healing.

Similarly, the study by Obied and Yahya (38) showed that licorice root extract led to a clear decrease in the ulcer index and a significant improvement in the histological structure of the stomach compared to the affected group.

In conjunction with the H&E results in this study, mucin loss in group (1) coincides with hemorrhage and inflammation, confirming that mucosal barrier degradation is the central factor in causing tissue damage. The omeprazole group (T2) showed a clear restoration of the PAS reaction, indicating improved mucin secretion, attributed to reduced acidity and protection of mucosal cells, consistent with the findings of group (T3). The purslane group showed moderate improvement, suggesting a limited ability to stimulate mucin secretion, while the licorice group (T4) exhibited a strong reaction, indicating a direct effect on mucus secretion stimulation. This can be explained by licorice's role in increasing prostaglandin production.

Mason's Trichrome

This revealed a near-complete loss of collagen fibers in the damaged T1 areas, with very few fibers remaining in the undamaged areas beneath them. This breakdown reflects the severity of the injury; the irritants have the ability to dissolve collagen fibers and destroy protective barriers, leading to tissue erosion. Furthermore, Aboulthana et al. (30) confirm that the absence of biochemical protection accelerates the degradation of collagen fibers due to the activity of proteolytic enzymes associated with acute inflammation.

This finding aligns with Park et al. (28) indicating that a normal gastric mucosa requires a cohesive collagen network (particularly types I and III) to provide structural support to the mucosa. This structural organization ensures the stability of the secretory functions observed in the control group. These results are consistent with Lanas and Chan's (39) study, which demonstrated that normal gastric mucosa is characterized by a mucin-rich layer that acts as a protective barrier against acid, pepsin, and pathogens.

Figure 11 shows the preservation of the surface layer and the integrity of the T2 basement membrane, with the observation of "mild fibrosis" appearing as thin strands of connective tissue. This mild fibrosis is a positive indicator of "quality of ulcer healing" (QOUH). According to Shin and Sachs (34) reducing the acid load gives cells the opportunity to initiate the repair fibrosis process. The 2014 study by El-Shinnawy et al. supports this finding, demonstrating that omeprazole not only protects myosin but also facilitates the remodeling of the collagen matrix, thus ensuring the restoration of tissue integrity.

Masson's Trichrome stained sections also showed a marked loss of collagen fibers in the damaged areas of the gastric mucosa, indicating deterioration of the extracellular matrix. The stained sections also revealed mild fibrosis in the form of fine strands of connective tissue, suggesting the initiation of tissue repair.

These results are attributed to the mechanism of action of omeprazole, which inhibits the proton pump (HKATPase) in the parietal cells of the stomach, leading to a reduction in gastric acid secretion and allowing for the healing of the damaged mucosa.

This T3 group exhibited different results (12), with destruction of the surface layer and random dispersal of collagen fibers in the basement membrane, indicating a loss of structural integrity in the affected areas. Although purslane showed "moderate" protection of mucin (PAS staining), Masson's staining results here suggest that its

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ability to protect "supporting fibers" was less efficient than omeprazole and licorice in this model. This observation is consistent with the findings of Uddin et al. (29), who suggested that the protective effect of purslane is primarily due to antioxidants that protect mucus-secreting cells, but may not be sufficient to prevent the mechanical degradation of deep fibers in severe injuries.

The histological sections of the purslane group T3, Figure (12), showed a relative improvement in the histological structure compared to the infected group, as the mucous layer and gastric glands appeared almost normal with an average reaction to PAS stain, indicating a partial improvement in mucin secretion. However, some histological sections showed destruction of the superficial mucosa layer and dispersion of collagen fibers, suggesting that the therapeutic effect of purslane was less compared to some other treatments.

These results are consistent with the study (26) which showed that purslane extract has a protective effect on the stomach due to its content of antioxidant compounds such as flavonoids and polyphenols, which work to reduce inflammation and improve the integrity of the gastric mucosa.

A study (34) also showed that purslane can reduce the severity of gastric ulcers and improve biomarkers associated with inflammation and oxidative stress in gastric tissue.

Licorice T4 (Figure 13) demonstrated a superior ability to maintain the integrity and order of the surface layer and basement membrane, with clear continuity in tissue structure. This result reflects the protective superiority of licorice. According to the study by Park et al., (28) licorice extracts increase prostaglandin levels, which not only increase myosin but also stimulate the growth of fibroblasts and the generation of strong collagen fibers that protect the basement membrane. This explains why the T4 group appeared more structurally cohesive compared toT3.

These findings are consistent with those of Wang et al. (20), which demonstrated that ulcer-causing agents such as ethanol or non-steroidal anti-inflammatory drugs lead to severe damage to the gastric mucosa, accompanied by bleeding, inflammation, and destruction of gastric glands, and that decreased mucus secretion is one of the most important factors leading to weakened mucosal barrier and the development of gastric ulcers.

These results are consistent with the study by Panji and Ghafouri (36) which showed that omeprazole treatment leads to a significant decrease in the ulcer index and a marked improvement in gastric histological structure. Another study demonstrated that proton pump inhibitors help reduce inflammation and promote gastric mucosal repair. These findings, as explained by Ganguly and Swarnakar (40), are consistent with research indicating that collagen damage plays a pivotal role in the development of gastric ulcers and delayed healing.

In comparison with the PAS results, the loss of collagen in (T1) is associated with the loss of myosin, indicating a double breakdown of the chemical (myosin) and structural (collagen) barrier, which explains the severity of the damage in this group.

In the omeprazole group (T2), mild fibrosis with preservation of the basement membrane was observed, representing an advanced stage of histological repair. The purslane group (T3) showed collagen fiber dispersion despite improved myosin, suggesting that its effect is more focused on surface protection than deep regeneration, which explains the discrepancy between the PAS and Masson results in this study. The licorice group (T4), on the other hand, demonstrated clear collagen preservation, indicating a high capacity to support extracellular matrix regeneration. This is attributed to the stimulation of fibroblasts and increased collagen synthesis, as indicated by Chen et al. (41)

Conclusion: The study concluded that the use of purslane and licorice extracts in the treatment of gastric ulcers had clear positive effects.

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Conflict of interest

The researcher declares that there is no conflict of interest regarding this work.

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