Cytoprotective Effects of Hydro-extracts of the Inner Bark of the Oak Tree Fruit (Jaft) on the Healing Process of Aspirin-Induced Gastric Ulcers in Rats

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Abstract
Introduction: A gastric ulcer is a mucosal lesion of the stomach that may occur after taking non-steroidal anti-inflammatory drugs such as aspirin, especially in cardiovascular diseases. The aim of this study was to evaluate the cytoprotective effects of the aqueous extract of oak (Jaft) against the mucosal damage effects of aspirin.

Methods: Seventy-two female Wistar rats weighing 200–240 g were prepared and divided into 6 groups. Groups I, II, and III received the Jaft extract at doses of 250 mg/kg/d, 500 mg/kg/d, and 700 mg/kg/d, respectively, and Group IV received omeprazole at a dose of 200 mg/kg/d. Groups V (patient’s control) and VI (healthy control) both received saline (0.9%) at 1 mL/kg/d. Mice were given aspirin (200 mg/kg/d) for inducing the gastric ulcer. After 14 days, they were anesthetized with ether, their stomachs were removed, and the blocks of tissue were prepared. The tissues were stained using the hematoxylin-eosin (H&E) dye and analyzed by the Olympus light microscope and OLYSIA software. Finally, IBM SPSS 26.0 software was used for statistical analysis of the data.

Results: The means ± standard deviation (SD) of the mucosal thickness in groups taking the Jaft extracts (250, 500, and 700 mg) decreased compared to the aspirin group (P<0.05). In addition, the mean ± SD of the mucosal gland thickness and mucosal folds in group V (with aspirin) decreased compared to the 250, 500, and 700 extract groups (P>0.05). The epithelial cell destruction, edema, venous congestion, and destruction of the capillaries of the mucosal and sub-mucosal areas in the extract groups decreased compared to the V group. Likewise, the number of mucosal cells in the gastric gland and the size of gastric parietal cells in the extract groups decreased compared to the V group.

Conclusion: This study generally elucidated the cytoprotective effect of the Jaft extract in the mouse model of gastric ulcer.

Keywords: Ulcers, Gastric ulcers, Quercus, Aspirin, Wound healing

Introduction
A gastric ulcer is a mucosal lesion of the stomach that occurs after high secretion of acid and pepsin, low mucosal blood flow, low synthesis of prostaglandin E2 and nitric oxide, and high serum levels of free radicals.1,2

According to the World Health Organization (WHO), one in every 10 Americans has suffered a gastric ulcer during their lifetime.3 The incidence rate of this disease in developed countries such as England was also estimated at 36%, which differed significantly from other gastrointestinal diseases such as esophagitis.4 The mortality rate was also reported closely to the 10% population of the world, with a high frequency in the elderly by age.5 Cardiovascular patients with a high intake of anticoagulants are at high risk.6 Because of non-specific gastric ulcer symptoms such as hemorrhage, its verification finally requires a clinical diagnosis such as endoscopy.4 However, taking non-steroidal
anti-inflammatory drugs such as aspirin, especially in cardiovascular disease, increases the risk of gastric ulcers.\textsuperscript{3,7} Aspirin application for the treatment of thrombotic cardiovascular diseases acts through the inhibition of cyclooxygenase-1, prostaglandin production, increasing free radicals, and infiltration of neutrophils into mucosal tissue, causing gastric ulcers.\textsuperscript{3,8} Gastric ulcer therapy is customarily performed with chemical drugs, including omeprazole, metronidazole, and ranitidine, which, in addition to its high cost, may be accompanied by side effects, autoimmunity, and relapse. Accordingly, much effort was made to find natural and herbal compounds with high efficacy and low cost in the treatment of gastric ulcers.\textsuperscript{3} Some of the scientific documents revealed that the extracts from the oak tree (bark, wood, and fruit) have therapeutic properties. This property is related to the tannin that prevents the growth of pathogens and accelerates wound healing.\textsuperscript{9-11} In addition to tannin, polyphenol compounds with antioxidant properties have been distributed in various plant species.\textsuperscript{12} Concerning the positive effects of the inner bark of the oak fruit on wound healing, we decided to investigate their effects on gastric ulcers induced by aspirin in the animal model.

Materials and Methods

Preparation of Plant Extract

This study used \textit{Quercus infectoria} and \textit{Quercus brantii} (an abundant tree in Lorestan province, west of Iran). The oak tree (\textit{Q. infectoria} and \textit{Q. brantii}; identified by \url{http://www.theplantlist.org}) was obtained from the Zagros Botanical Garden, Khorramabad, Lorestan, Iran, and identified by a botanist working in the Medicinal Plants Research Center of Sistan and Baluchestan College, Zahedan, Iran. The plant had gray-green leaves, fluffy branches, and oval fruits. A mixture of the chopped bark of the oak fruit was prepared, which is depicted in Figure 1a.\textsuperscript{13}

The chopped bark of the oak fruit (1 kg) was mixed, then soaked in distilled water (4 L, 48 hours), and filtered using filter paper. The obtained extract was evaporated using a rotary evaporator (50 °C), and the residue was poured into a Pyrex glass container and placed in the incubator (40–45 °C) until the dry extract was prepared.\textsuperscript{3} The dry extract was weighed and stored at 4 °C.\textsuperscript{14}

Animals and Prescription of Extract

 Seventy-two female Wistar rats (weighing 200–240 g) were obtained from the animal house of Zabol University of Medical Sciences and kept in suitable conditions (taped water, sufficient food, and light) for ten days.

Mice were randomly sorted into 6 groups of 12 each. The Roll of the die method was used for randomization. The six-sided die contained numbers 1–6, which were specified for the 6 study groups. Groups 1–5 suffered from aspirin-induced gastric ulcers, and the sixth was a healthy control group. The first, second, and third groups received the aqueous extract at doses of 250 mg/kg, 500 mg/kg, and 750 mg/kg daily for 14 days, respectively. The fourth group received omeprazole (200 mg/kg). Each of the fifth and sixth (healthy control) groups received physiological serum (saline 0.9%, 1 mL/kg).

Induction of Gastric Ulcer

Mice were starved for 48 hours. For the prevention of dehydration, mice were fed with an 8% sucrose solution in 0.2% sodium chloride.\textsuperscript{15} Then, they were given aspirin (200 mg/kg/d) dissolved in a 1 mL solvent (carboxymethyl cellulose 1%) for three days.\textsuperscript{16} For the verification of the induction of gastric ulcers at the end of the study, they were anesthetized with ether and killed. The number of spotted lesions of the stomach was counted according to the protocol suggested by Pandit et al.\textsuperscript{17} Spot lesions with 1 mm dimensions were counted, and every 5 spot lesions were considered 21 mm wounds.

![Figure 1. The Chopped Bark of the Oak Fruit: (a) Oak Fruit, (b) Cupule, (c) Oak Fruit Without Cupule, (d) Seed Coat (Testa), (e) Fruit Wall (Pericarp), and (f) Cotyledons](image-url)
Sampling
Three hours after the last prescription of the extract dosage, the mice were anesthetized with ether and then dissected. Their stomachs were removed, washed with saline (0.9%), kept in formalin (10%) for 24 hours, and then transferred to formalin (5%) for 24 hours. The tissue slices (0.5 × 0.5 mm<sup>2</sup> thickness) were prepared and placed in the tissue processing machine. After the preparation of tissue blocks, incision (5-μm thickness) sections were prepared using a rotary microtome. The tissues were stained with the H&E dye and analyzed using the Olympus light microscope and OLYSIA software.

Statistical Analysis
Data were analyzed using IBM SPSS 26.0 statistical software (IBM Company in Chicago, USA). The descriptive data were statistically analyzed using the mean ± SD. The distribution (normal and non-normal) of data appeared by the Kolmogorov-Smirnov (K-S) test (P< 0.05). Subsequently, one-way analysis of variance and post-hoc Tukey’s tests were used to compare the mean of the data. A P value of< 0.05 was considered significant.

Results
Quantitative Histometric Analysis
The mean ± SD of the wound area of the aspirin group (1.90 ± 0.07) increased compared to healthy control groups (0), extract groups 250 (1.4 ± 0.05), 500 (1.04 ± 0.09), and 700 (0.260 ± 0.05), and the omeprazole (1.34 ± 0.05) group (P< 0.05, Figure 2a).

The mean ± SD of the mucosal gland in the aspirin group (521.19 ± 16.80) did not differ (P>0.05) compared to the extract groups 250 (627.86 ± 21.05), 500 (623.34 ± 21.05), and 700 (657.62 ± 11.39). However, its difference was significant compared to the healthy control (998.58 ± 24.51) and omeprazole (482.62 ± 17.92) groups (P< 0.05, Figure 1b).

The mean ± SD of the mucosal fold thickness of the aspirin group (673.77 ± 30.25) did not differ compared to the extract groups 250 (681.73 ± 24.74), 500 (707.85 ± 22.19), and 700 (713.19 ± 26.57), and the omeprazole (693.73 ± 17.25) group (P> 0.05, Figure 1c).

The mean ± SD of sub-mucosa thickness in the aspirin group (2129.99 ± 81.05) increased compared to the healthy control group (62.70 ± 4.95), the extract groups 250 (176.85 ± 7.51), 500 (152.36 ± 2.81), and 700 (105.63 ± 3.74), and the omeprazole (175.57 ± 8.19) group (P< 0.05, Figure 1d).

Histological Evaluation
The histological analysis of the aspirin group elucidated the disintegration of the tissue structure of the stomach (Figure 3). The destruction of the epithelial lining and increase of edema in the sub-mucosal area, venous congestion, and destruction of the capillaries of the mucosal and sub-mucosal areas were the causes of mucosal bleeding (Figure 3b). The sites of inflammation in the gastric mucosal tissue were observable in the aspirin group (Figure 3b).

The results demonstrated apoptotic bodies, infiltration of leukocytes, a decrease in the thickness of the gastric gland, and gastric mucosal folds. In addition, the venous...
congestion of the mucosal area and mucosal folds represented an increase. These findings in the extract groups (250, 500, and 700) were less and close to normal compared to the aspirin group (Figure 4a, b, and c).

The mucosal cells of the gastric gland in the extract groups (250, 500, and 700) decreased compared to the aspirin group. Similarly, the number and size of gastric parietal cells (acid-secreting) decreased in comparison to the aspirin group (Figure 4a, b, and c).

**Discussion**

Despite the apparent differences between humans and mice, there are extensive similarities, especially in the genomic sequences, anatomical structure and morpho-functionality of the digestive tract, and normal flora bacteria in the digestive tract of both species.\(^{18-20}\) Therefore, rats were used in this study.

In our study, aspirin (200 mg/kg) was utilized for three consecutive days to induce experimental gastric ulcers in mice. This protocol was validated before by Abushwereb...
and Tolba, and Wang et al.\textsuperscript{21,22} For the examination of the structural tissue, the H&E staining assay was employed, which was orchestrated with the study by Wang et al.\textsuperscript{22} Our findings demonstrated the damaged mucous tissue and damaged cells that indicate successful induction of the gastric ulcer in the mice (Figure 3b). However, the absence of this condition in the extract groups (250, 500, and 700) confirmed its protective effect against disease induction (Figure 4a, b, and c).

Our findings showed that the mean ± SD of the wound area in the aspirin group significantly increased compared to other groups. Based on the results, the mean ± SD of the mucous gland thickness in the aspirin group decreased compared to 250, 500, 700, and the healthy control group. However, it increased compared to the omeprazole group.

In line with our study, Li et al examined the mucosal area of a gastric ulcer experimentally and allocated grades 1 (the lowest) and 10 (the worst) macroscopically and based on the severity of the ulcer.\textsuperscript{23} Our findings represented a decrease in the size of the mucous gland quantitatively, which results in bleeding.

Our study manifested that the mean ± SD of the mucosal fold thickness of the aspirin group was reduced compared to the 250, 500, 700, and omeprazole groups. The folds were flattened and removed. However, in the animal model of gastric ulcers induced by stress, Barmak et al showed that the mean ± SD of the mucosal gland thickness (µm) in the groups treated with the Jaft extract in a dose-dependent manner increased in comparison to the patient and healthy control groups. In addition to the edema and venous congestion in the sub-mucosal layers, a decrease in the length of mucosal glands and folds was observed in patient control group.\textsuperscript{24} Abushwereb and Tolba also analyzed the effect of the Artemisia herba-alba leaf extract on the experimental gastric ulcer. They manifested that a dose of 10 mg/kg 30 minutes before the induction had the most protective effect. They evaluated the number, length, and size of gastric ulcers.\textsuperscript{25} Another study by Wasman et al evaluated the preventing effect of Polygonum minus leaf extract on the experimental model of the gastric ulcer and revealed that the folds of the gastric mucosa decreased and were flattened. In addition, they reported a decrease in venous congestion, leukocyte infiltration, and edema in the sub-mucosal areas compared to the patient control group.\textsuperscript{25} The histological analysis of our study uncovered the disintegration of the tissue structure of the stomach by aspirin. However, there was a gradual reduction in the destruction of the epithelial lining, edema, venous congestion, destruction of the capillaries of the mucosal and sub-mucosal areas, and bleeding in the mucosal area in the groups treated with the extract in comparison to the aspirin group. Barmak et al confirmed an increase in edema and venous congestion in the sub-mucosal layers of the patient control group,\textsuperscript{24} which conforms to our findings. Studies by Wasman et al and Al Batran et al for the pathological examination of the gastric tissue in mice suffering from gastric ulcers as the control group demonstrated edema, severe epithelial damage, the epithelial lining disruption of the gastric mucosa, and neutrophils infiltration into the sub-mucosal area compared to the extracted groups. Moreover, at high doses of the extract, it was significant.\textsuperscript{25,26} Microscopic analysis of mucosal tissues showed a decrease in the surface cells and mucosa in the epithelial lining of the neck gastric glands in the aspirin group. The number and size of parietal cells (acid secretors) also increased significantly compared to the extract groups. In their study, da Silva Prado et al reported that the plenty of tannins in the plant extracts (oak bark) caused an increase in epithelial protective thickness against harmful agents such as aspirin, ethanol, and indomethacin. Tannin causes a high secretion of mucus and is conjugated to it, increasing epithelial thickness.\textsuperscript{27} This study did not examine the antioxidant and antibacterial properties of the Jaft extract; nevertheless, numerous in vitro studies had previously confirmed these functions.

For instance, Umachigi et al revealed the efficacy of the oak leaf extract (800 mg/kg) in the experimental model for skin wounds. Their findings demonstrated an increase in the antioxidant activity rate of catalase and superoxide dismutase and wound healing.\textsuperscript{28}

Investigations by Khennouf et al also confirmed that tannin with oak leaf origin (in a dose-dependent manner) prevents the peroxidation of free fatty acids in gastric ulcers.\textsuperscript{29} Furthermore, the activity of the H/K ATPase pump in gastric ulcers was ameliorated after the application of tannin.\textsuperscript{30} Safary et al also determined that the oak leaf extract inhibited the growth of Gram-negative bacteria (E. coli, Proteus, Shigella, and Salmonella).\textsuperscript{31}

**Conclusion**

The findings generally elucidated the cytoprotective effect of the Jaft extract in the mouse model of gastric ulcers induced by aspirin. The decrease in the mean ± SD of the wound area and an increase in the mean of the mucosal gland thickness in the extract groups clarified the more protective effects of the Jaft extract. Moreover, lowering the leukocyte infiltration and gastric mucosal folds, plus the decrease of edema and improvement of mucosal and sub-mucosal bleeding, were the signs of the protective efficacy of the Jaft extract in the experimental gastric ulcer. To reveal the other characteristics of the Jaft extract, it is suggested that future studies evaluate the gastric mucosal contents in terms of pH, prostaglandin E2, the serum level of malondialdehyde, oxidant, oxygen, and nitrogen free radicals, and the activity of the antioxidant enzymes (Catalase, superoxide dismutase, and glutathione reductase).
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Authors’ Contribution

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Competing Interests

The authors have no conflict of interests.

Ethical Approval

This study was approved by the Research Committee of the Faculty of Medicine on October 18, 2022, and obtained ethical code (IR. ZBMU.AEC.1402.002) from the Expert Committee on Ethics in Working with Laboratory Animals, Zabol University of Medical Sciences, on May 2, 2023.

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