Original Article

Efficiency Of Wharton's Jelly Mesenchymal Stem Cells With Saccharum Officinarum Juice And Glycyrrhiza Glabra Extract In Modulation Of Histopathological And Physiological Changes During Gastric Wound Healing In Rats

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Abstract

Objective : Peptic ulcer disease means some kind of benign damage to the mucosa and the lining of the digestive tract. There is usually a link between gastric acid secretion and this injury, but nowadays, the leading cause of these ulcers is the presence and proliferation of Helicobacter pylori bacteria. Given the effects of chemical drugs, medicinal plants, and cell therapy as an alternative to treating diseases seems to be appropriate.

Methods: Twenty-four male Wistar rats weight 300±2 g randomly divided into six groups(n=4), control, peptic ulcer (PE) by Acetic acid, PE+ Saccharum officinarum juice as drinking water (n=4), PE+ Glycyrrhiza glabra extract (Gge) 200 mg/kg, PE+ distilled water as gavage control, PE+ Gge 200 mg/kg+ Soj + Wharton's jelly stem cells (WJMSCs). After 14 days, all groups evaluated and compared factors such as histopathological studies, weight and acidity changes, and changes in blood serum cytokines.

Results: According to the results of this study, the groups receiving Soj and Gge had a significant difference in the mentioned factors Compared to the control group ($P<0.05$). Although WJMSCs, when combined with Soj and Gge, had a significant effect on inflammatory factors and body weight changes ($P<0.05$), they were able to increase the thickness of the stomach wall.

Conclusion : These results suggest that Soj and Gge have given their compounds, which can be considered an alternative to gastric ulcer treatment, which can be accelerated and more efficient with cell therapy.

Keywords: Mesenchymal Stem Cells, Saccharum officinarum , Glycyrrhiza glabra , Gastric Wound , Rat

1- Introduction

Gastric or peptic ulcer is a benign lesion of the mucosal epithelium that results from overexposure to the aggressive activity of pepsin or acid [1]. Under normal conditions, a physiological balance exists between stomach acid secretion and natural gastroduodenal mucosal defense. Peptic ulcer, an eroded part of the gastric mucosa, may occur when the above-mentioned physiological balance is disturbed [2]. Increasing our knowledge about the pathogenesis of this disease has changed the treatment approach from an acid-based disease to an infectious disease and has made studies to manage this disease more accurate. Identification of Helicobacter pylori (H. pylori) has attracted attention. Importantly, studies have shown that killing this bacterium is very important in reducing the symptoms of peptic ulcer disease [3]. The prevalence of this disease in the general population is estimated to be around 5 to 10%, but fortunately today's studies indicate a reduction in the incidence and mortality of this disease [4].

Helicobacter pylori, consumption of ethanol, acids, bile salts, pepsin secretion and some drugs such as nonsteroidal anti-inflammatory drugs, syndrome A, mucosal cell damage, impaired mucin secretion and antioxidant enzymes, lipid peroxidation and as the main pathogens Stomach are considered [5].

Studies have shown that 80% of gastric ulcers and 90% of duodenal ulcers are caused by Helicobacter pylori [6]. In Western countries, due to the control and reduction in the prevalence of this bacterium, drugs, especially NSAIDs and acetylsalicylic acid (ASA) [7]. Also, stress, smoking, and spices are known to be major contributors to the disease [2,8-9].

There are several models of medicine for the treatment of gastric ulcer, but unfortunately each of them has different interactions and side effects. The existence of such medicinal disorders has led to studies focusing on medicinal plants with less side effects. In traditional medicine, there are many plants that have specific properties to deal with various types of wounds, or at least have compounds that can be chemically modified. Used as an anti-ulcer compound. It should also not be forgotten that herbal medicines are much cheaper. Therefore, the use of herbal medicines, if effective, can be used as a suitable option for wound management [10].

Glycyrrhiza glabra (G.glabra), from the family Leguminosae, is a plant that is mainly native to Egypt and other countries. The root of this plant has medicinal properties and high nutritional value and is mainly used as a cold drink and in the preparation of some medicinal compounds as a sweetener [1]. HPLC evaluation of the root of this plant has shown that it contains various compounds such as flavonoids, triterpenes, saponins, different types of sugars and compounds such as coumarin. So far, studies have been performed on the extract and active ingredients of G. glabra, which have shown that they have specific pharmacological effects, including the treatment of bronchitis, rheumatism, Addison's disease, gastric ulcer and allergic conditions, and chronic type B and C hepatitis [11].

Saccharum officinarum (S. officinarum), sugarcane, is a species of sugarcane genus, from cereals and natives of warm temperate regions to the tropics, and is used in various sectors to produce sugar products [12]. Sugarcane has had many uses since ancient times. Its use for the production of sugar derivatives and alcohol production has been used in the livestock industry as animal feed and in traditional medicine in various cases [13].

Medical science seeks to find alternative therapies for the treatment of gastric ulcers. To this end, finding molecular pathways in the healing process of gastric ulcer is very important [14]. One of the newest treatments is the use of mesenchymal stem cells or platelet-rich plasma, which have very important effects on the control of cytokines and growth factors as important factors in the healing process of gastric ulcer [15]. The main source for Wharton Jelly (WJ) stem cells is the umbilical cord [16]. These cells with specific properties such as excellent adhesion are introduced as special cells for treatment. On the other hand, it seems that embryonic mesenchymal stem cells have better expression and efficiency than adults [17-18].

The present study examined whether topical transplantation of WJMSCs accelerated gastric ulcer healing. We also explored the effect of S. officinarum juice and G.glabra extract with WJMSCs on gastric ulcer healing.

2- Materials and methods

The study was approved by the behavioral laboratory of the faculty of basic sciences, college of the Shahid Chamran University of Ahvaz, Ahvaz, Iran.

2-1- Experimental animals

Twenty-fore male Wistar rats weight 300±2 g were purchased from the animal house of the biology department, university of Shahid Chamran (Ahvaz, Iran). Animals were housed and maintained under standard conditions (12 h light/dark cycle, 23 ± 1 "c). Food (pellet from pars feed, Iran) and distilled water were given. The animals were cared for following the recommendations.

2-2- Extracts of plants

2-2-1- Glycyrrhiza glabra

The roots of G. glabra were purchased from the Medicinal Plants Stor, Ahvaz, Iran. Botanical identification of the plant was made of the herbarium sections in the department of botany university of Shahid Chamran of Ahvaz. The dried roots and rhizomes were crushed and extracted with 70% v/v ethanol using Soxhlet's apparatus. The hydroalcoholic extract of G. glabra was pooled and concentrated under reduced pressure and evaporated in the air to dry. The extract was stored in a refrigerator and reconstituted in water for injection [19]. Previously, the compounds are reported with the HPC technique [20].

2-2-2- Saccharum officinarum

S. officinarum from Haft-tappeh farms in Khuzestan, Iran, was selected for the juice. Briefly, S. officinarum was chopped, and then the juice was extracted in a juice-making machine (PSJ 50, Tehran, Iran.). Finally, the juice was diluted in drinking water. Previously, the compounds are reported with the HPC technique [21]. The juice was used as drinking water in the treatment period.

2-3- Grouping of animals

The animals were randomly divided into six groups, control (n=4), peptic ulcer (PE) by acetic acid (n=4), PE+ S. officinarum juice as drinking water (n=4), PE+ G. glabra extract 200 mg/kg [21] (n=4), PE+ distilled water as gavage control (n=4), PE+ G. glabra extract 200 mg/kg+ S. officinarum juice+ WJMSCs (n=4).

2-4- Induction of gastric ulcers

All mice were first deprived of food for 24 hours to induce gastric ulcer, but they were given water. Two hours before surgery, the water of the mice was discontinued to allow their digestive tract to be completely empty. The rats were then anesthetized with Ketamine/Xylazine (100/10 mg/kg) intraperitoneal anesthesia. After anesthesia, the mice were placed on the right side and from the left side (almost in the middle of the body). After that sheave and sterilizing with alcohol and betadine, a small incision was made in the abdomen, so the stomach was removed so that it was not screwed. A clamp blocked two stomach heads, and a 0.25 ml 60% acidic solution was injected with an insulin syringe into the trunk of the gut region without injection into the vein.

After 45 seconds, the same syringe removed the acid, and the stomach was twice infused with normal saline Washed. The stomach was restored, then peritoneum and skin were sutured, and the mice were placed on the abdomen and kept in a cage separately [22]. One day after surgery, experimental groups were studied for 14 days. Eventually, after 14 days, animals were killed by a method of Athanasia for histopathological studies.

2-5- Preparation of Wharton's jelly cells

Human WJMSCs were derived as previously reported [14]. Briefly, the human umbilical cord was harvested from full-term infants during delivery. The cord blood was immediately drawn and dissected into pieces of 1 cm following washing. Cells were isolated by an explant technique so that each piece was slit opened carefully with a scalpel, and the vessels were removed and surrounding jelly collected. Then the jelly was cut into pieces approximately 3 mm, placed onto cell culture flasks, and incubated for 2 weeks to allow cell migration and proliferation. DMEM low glucose medium supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin and 100 mg/ml streptomycin were used, and this medium was changed every 2 days. The cells were passaged by 0.25% trypsin/EDTA (Figure 1).

Figure 1- Preparation steps of Wharton's jelly cells. A. Explant method for isolating Wharton jelly stem cells 10 days after culture. B-Wharton jelly stem cells were isolated in the first passage. C. Wharton Jelly Stem Cells Isolated in Third Passage. All samples were taken with a 20X lens

2-6- Histopathological studies

After the autopsy, the stomach samples were prepared and fixed in 10% formalin buffer. For preparation of tissue sections after preparation, they were cut by a rotary microtome with a thickness of 5 µm and stained with the hematoxylin-eosin technique. Stomach samples were studied in different groups at different magnifications for qualitative and quantitative studies. Mucosal thickness was measured in specimens obtained by a graded lens (20x) and 10x magnification. For this purpose, each sample was randomly sampled from 4 involved areas, and 16 data in micrometer were recorded in each group [23].

2-7- Bodyweight studies

At the beginning and the end of the study, the weight of the animals was evaluated after 14 days and compared.

2-8- Collection of gastric juice

At the end of the experiment protocol period (2 weeks), the rats were subjected to 48 hours of fasting, and then they were weighed and sacrificed by cervical dislocation. A midline abdominal incision was done, the stomach was ligated at the lower esophageal sphincter, and 2ml of saline (pH=7.0) was infused in the stomach through the pylorus. Then the gastric content was collected for acid titration. An automatic titrator radiometer titrated gastric washout (1 ml) against 0.01N of NaOH to endpoint 7.0, and acid content was expressed as mEq H+ [24].

2-9- Cytokine determination

Blood serum cytokine levels (IL-6, IL-7) were evaluated by ELISA using reagents and instructions from the Biotrak ELISA System.

2-10- Evaluation of disease activity index (DAI)

The DAI score was determined by combining scores of:

i) Body weight loss,
ii) stool consistency, and
iii) fecal occult as previously described [11].

The average of the three values was defined as the DAI. The body weight recorded on day 0 was considered the baseline. Bodyweight loss was calculated as the percent difference between the initial body weight at baseline and the bodyweight on the measurement day [25]. The graphical summary of the experiment is shown in Figure 2.

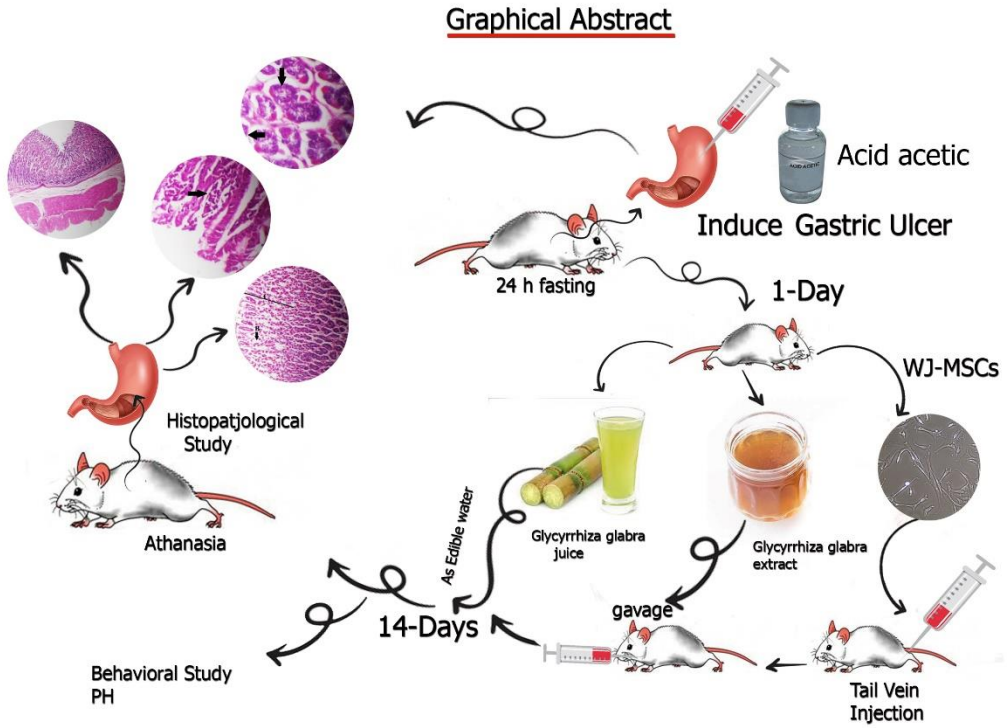

Figure 2-Graphical summary of the experiment.

3- Results

3-1- Bodyweight changes

The mean weight changes of rats in S.officinarum juice and G. glabra extract groups were significantly higher than in the gastric ulcer group ($P<0.01$), also WJMSCs, when combined with S.officinarum juice and G. glabra extract, had a significant effect ($P<0.01$) (Figure 3).

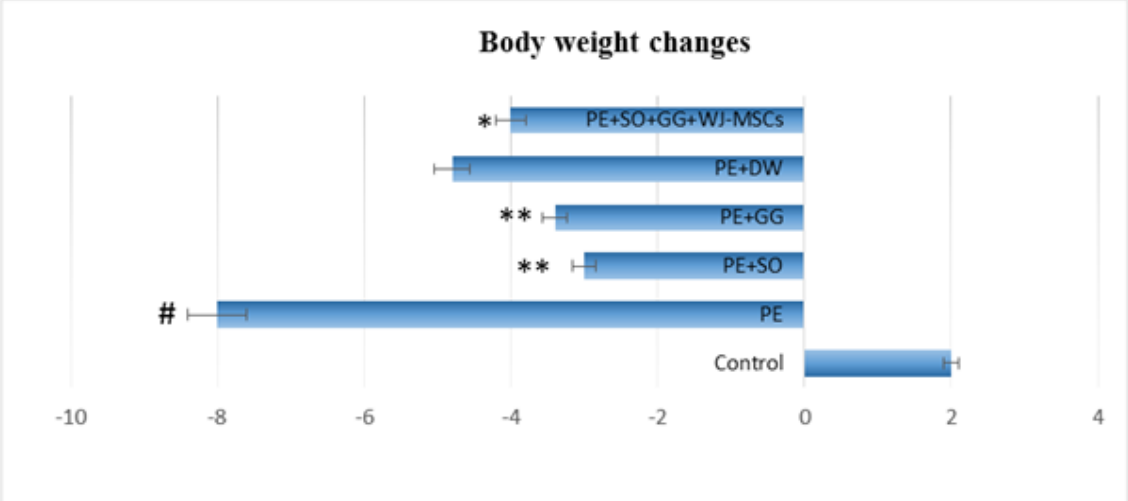

Figure 3-Body weight changes in all groups (gram). PE: peptic ulcer, SO: S. officinarum juice, GG: G. glabra extract, DW: distilled water. # P<0.05 compare to control, **P<0.01 compare to PE, *P<0.05 compare to PE, n=4.

3-2- Acidity Levels

Measurement of gastric secretions showed a significant difference in the S. officinarum juice (P<0.01) and WJMSCs+ S. officinarum juice+ G. glabra extract group compared to the other groups (P<0.05) (Figure 4).

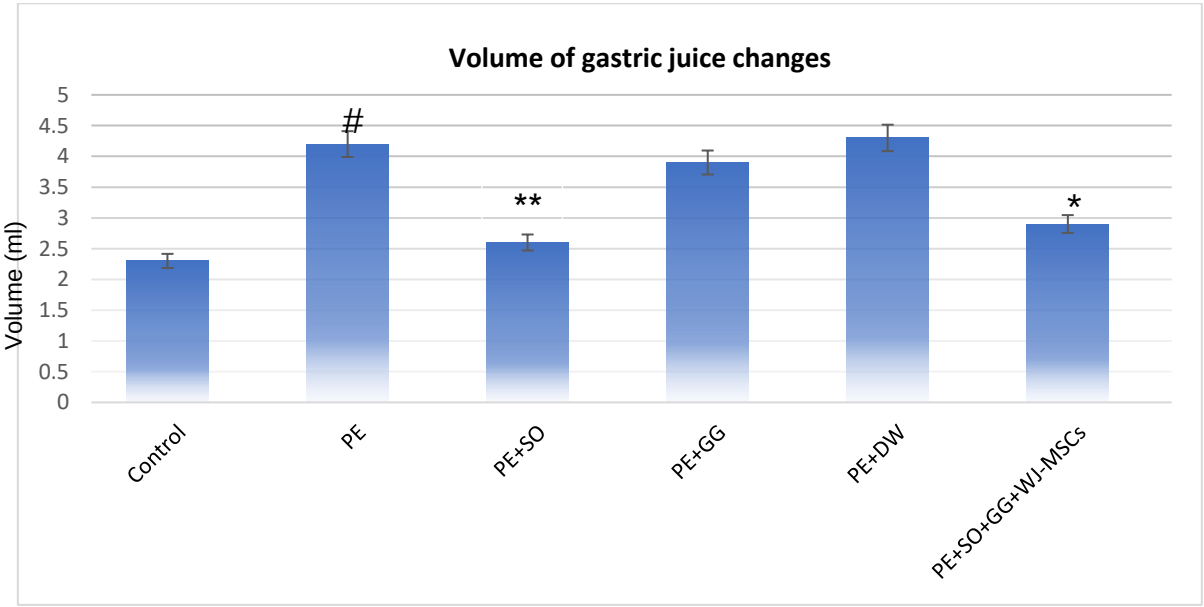

Figure 4- Volume of gastric juice changes in all groups (ml). PE: peptic ulcer, SO: S. officinarum juice, GG: G. glabra extract, DW: distilled water. # P<0.05 compare to control, **P<0.01 compare to PE, *P<0.05 compare to PE, n=4.

However, the acid content in different groups showed that the groups receiving S. officinarum juice and G. glabra extract showed a significant difference compared to the negative control group (Peptic ulcer) (Figure 5) (P<0.05).

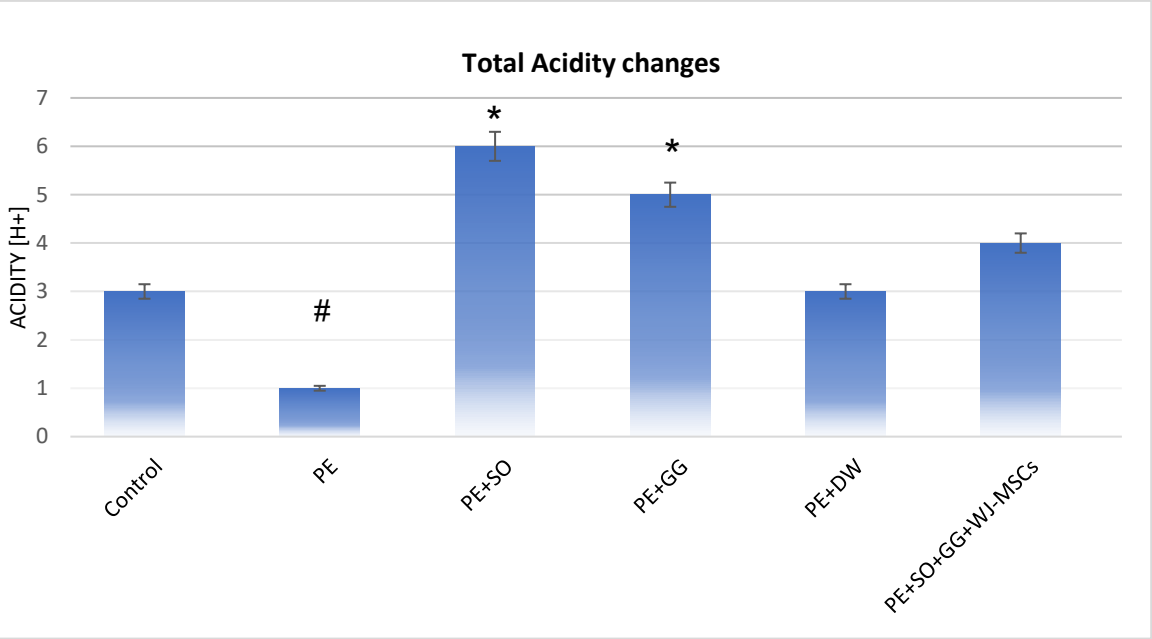

Figure 5- Total Acidity changes in all groups ([H+]). PE: peptic ulcer, SO: S. officinarum juice, GG: G. glabra extract, DW: distilled water. # P<0.05 compare to control, *P<0.05 compare to PE, n=4.

3-3- Histomorphometric

The results of mucosal thickness measurements in these specimens are summarized in the following tables (Tables 1 and 2).

Table 1: Analysis of variance (mean squares) of the mucosal thickness (µm) in study groups

average of squares		
Sources of changes	Degrees of freedom	**Stomach**
Treatment	5	***762581.092**
Trial error	18	**47016.124**
Coefficient of variation(%)		**5/7**

*: Significant at the 5% probability level

Table 2: Comparison of mean mucosal thickness in the study groups

Stomach	study groups
902^{B}	Negative control (healthy)
250^{A}	Positive Control (Experimental Wound)
528^{A}	Saccharum officinarum juice
992^{B}	Glycyrrhiza glabra extract
446^{A}	Saccharum officinarum juice + Glycyrrhiza glabra extract
1440^{C}	Saccharum officinarum juice + Glycyrrhiza glabra extract + WJ-MSCs

Although gastric mucosal thickness increased in the treatment groups (histomorphometrically) compared to the positive control group, this increase was only in the sixth group (S. officinarum juice treatment, G.

glabra extract, and stem cells) and the third group (treatment group). G. glabra extract was statistically significant (P<0.05).

3-4- Histopathological results

Microscopically, the negative control group did not have any pathological findings (Figure 6-A). In the positive control group (experimentally wound), necrosis of the epithelial cells at the top of the mucosa, gastric gland destruction was observed in the Parietal (Figure 6-B).

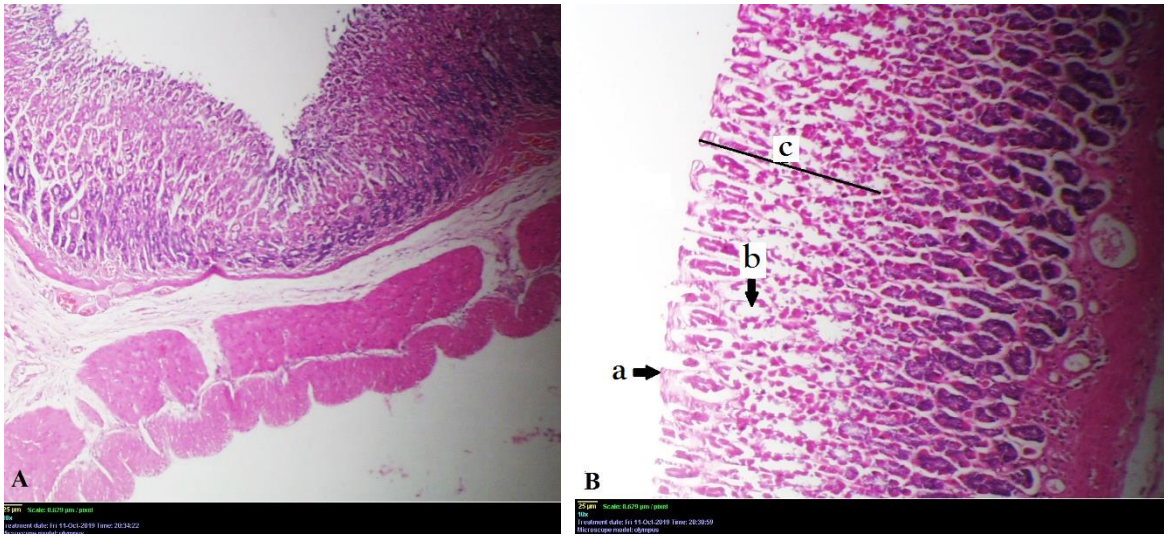

Figure 6– A: Gastric stomach, in a negative control group sample. B: Glandular stomach, in one rat positive control group (experimentally wound). a: necrosis of gastric epithelial cells, b: the destruction of gastric glands, and c: shows the necrotic area (H&E staining).

In the two groups treated with S. officinarum juice and G. glabra extract, different degrees of necrosis of the epithelial cells, drainage, and atrophy of the gastric mucosa were observed in the gastric mucosa-treated group (Figure 7-A), as well as the accumulation of defense cells in gastric tissues was observed (Figure 7-B).

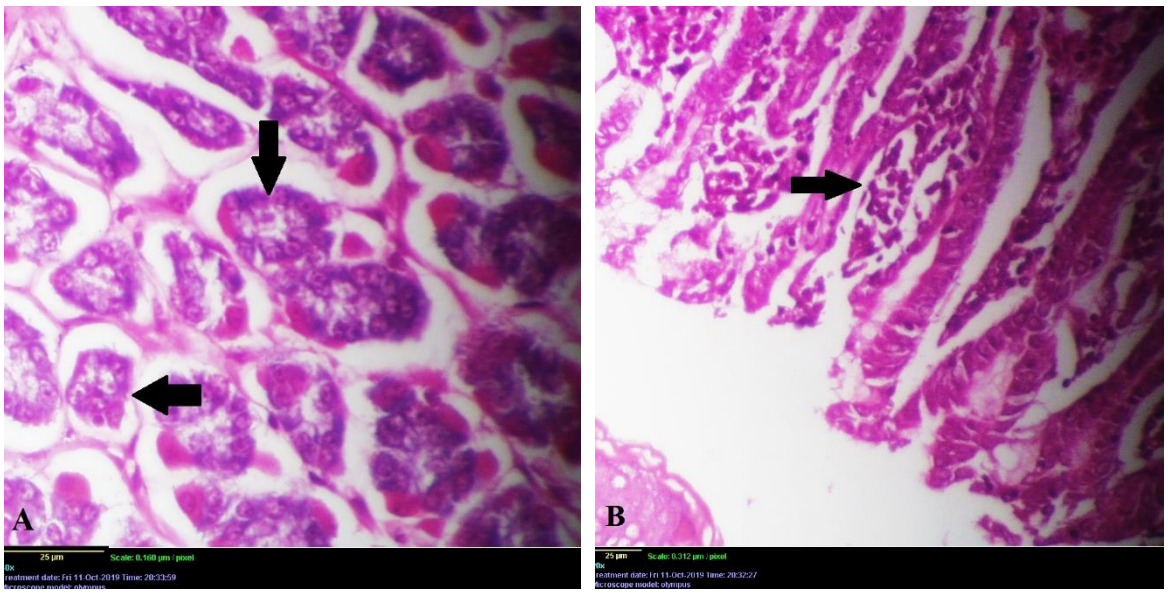

Figure 7-A: Stomach gland in a rat head treated with licorice extract. Black arrows indicate gastric atrophy. B: Gastric gastric ulcer in one rat in the cane-treated group. The black arrow indicates the presence of defense cells inside the gastric tissue (H&E staining).

Also, microscopic examination of the stomach of the last group of mice (S.officinarum juice, G. glabra extract with stem cell infusion) did not significantly increase gastric mucosa thickness.

3-5- IL-6 and L-17A Determination

Compared to the negative control group (Peptic ulcer), all groups showed significant differences in IL-6 and IL-7A. However, the WJMSCs+ S. officinarum juice+ G. glabra extract group showed significant differences only in both IL-6 (Figure 8) ($P<0.05$). It indicates the healing effects of these compounds on the gastric ulcer.

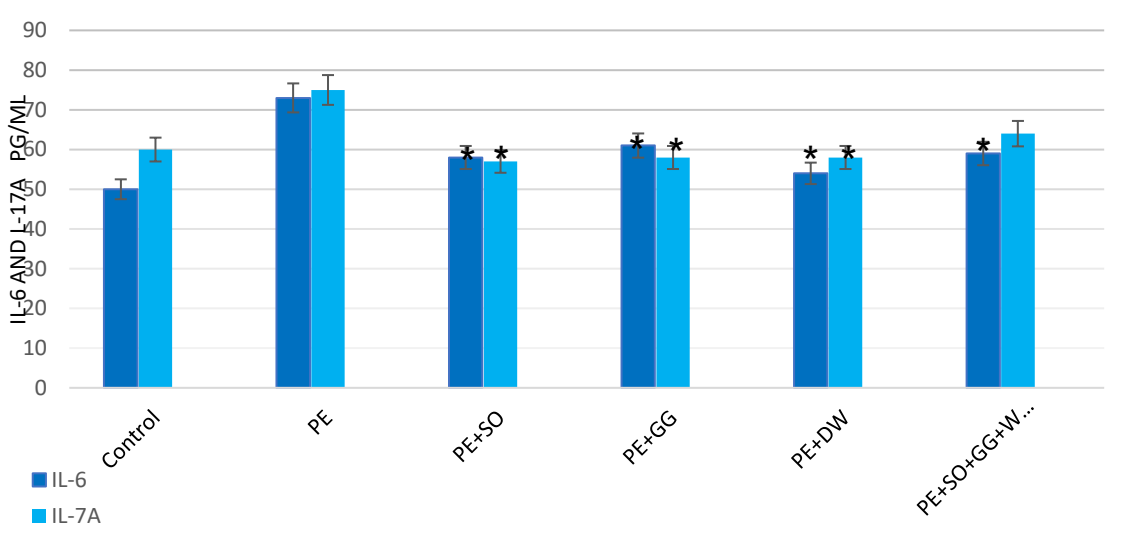

Figure 8- Cytokine determination in all groups. PE: peptic ulcer, SO: S. officinarum juice, GG: G. glabra extract, DW: distilled water. *$P<0.05$ compare to PE, n=4.

4- Discussion and Conclusions

In the present study, using S. officinarum juice for 14 days improved the histomorphometric indices, reduced gastric acid, and decreased blood serum cytokines. Also, G. glabra extract had a similar effect. It indicates the anti-inflammatory properties of gastric ulcer. When the two compounds were combined, the effects of each other were somewhat reduced. The use of WJMSCs, when combined with S. officinarum and G. glabra, had a significant effect on inflammatory factors and body weight changes ($P<0.05$). However, they were able to increase the thickness of the stomach wall.

Studies have shown that compounds found in S. officeinarum extract can increase mucus production in gastric lining cells by up to 70%, possibly by increasing glutathione [26].Glutathione is one of the most important protective mechanisms against ulcer development in the stomach. This combination reduces superoxide dismutase (SOD) and catalase (CAT), thus playing an important role in reducing gastritis [27].

Due to the anticoagulant effects of S. officeinarum extract on the parameters affecting wound initiation and induction, this plant may be considered a highly desirable anti-ulcer. It has gastric acid-neutralizing and protective properties comparable to the reference drug. A study has shown that ethanolic and methanolic leaf extracts of S. officeinarum have wound healing properties against experimental wounds in mice. This study confirms folkloric claims about the benefits of S. officeinarum in wound healing. Phytochemical analysis of Saccharum officinarum extract showed the presence of carbohydrates, flavonoids, and glycosides in both extracts. Therefore, the anti-ulcerative properties of Saccharum officinarum leaf extract may be attributed to active principles such as flavonoids, glycosides [28].

According to a study of a phenolic extract of S. officinarum juice, the protective effect against in vivo toxicity showed potent inhibition of lipoperoxidation, indicating strong antioxidant effects of cane extract [29]. In one study, the anti-inflammatory effects of S. officinarum extract were investigated, and the sugarcane extract had anti-inflammatory properties. These anti-inflammatory effects have been reported due to inhibitory effects on arachidonic acid metabolism [30].

S. officinarum juice can be considered a rich source of antioxidants. It is rich in apigenin, which is known to be a potent inhibitor of free radical lipid peroxidation [29]. Numerous studies have shown that licorice extract or its derivatives have diuretic, laxative, sedative, antipyretic, antimicrobial, anti-anxiety, anti-virus, anti-inflammatory, and antioxidant activities [31]. Studies have shown that licorice extract has antioxidant activity and protects tissue against free radicals. The antioxidant property of flavonoids and tannins is related to the anti-ulcer activity because free radicals are produced in gastric mucosal lesions. Flavonoids have shown cellular protection activity in different models. It has recently been shown that licorice in a combined product can heal wounds effectively [32]. Licorice has been reported to contain several flavonoids, alkaloids, and many other chemicals responsible for its effects. Several flavonoids found in various plants have reduced gastric ulcer formation. Therefore, it is hypothesized that flavonoids may be at least partially involved in the anti-ulcer effects of the stomach. Apart from flavonoids, licorice also contains steroids such as beta-sitosterol, which effectively reduces the progression of gastric ulcers. Glycyrrhizinic acid found in licorice extract has also been shown to have ulcerative gastric properties [33-34]. Also, the study results showed that licorice extract could partially control and reduce Helicobacter pylori and reduce its complications in gastric ulcer and improve gastric ulcer. Our findings showed that licorice could be suggested as an alternative to quadruple therapy when this regimen is not available. At the same time, licorice is low cost, highly tolerant, and with minimal side effects [35].

MSCs are a subset of adult somatic stem cells that reside in the bone marrow and many organs. These cells are regenerative because of their multiplicity in medicine. Mesenchymal stem cells are injected directly into the damaged heart or liver and improve tissue repair and organ function [22]. However, little is known about the therapeutic effects of MSC on gastrointestinal disorders. The results of one study showed that the use of hematopoietic stem cells (HSCs) in gastric ulcers induced in rats could improve wound healing, indicating a good condition. This study showed that HSCs or cells might secrete growth factors such as vascular endothelial growth factor (VEGF). Also, proteins such as 2 and A1 in these cells, TGF-β, and hepatic growth factor (HGF), which participate in the process of gastric ulcer healing, maybe the result of the activity of these cells, indicating the effect of these cells on healing stomach ulcers [36].

Studies have shown that angiogenesis is an important process in healing gastric ulcers. On the other hand, VEGF is a critical factor in the angiogenesis process. Studies have shown that MSC stem cells induce VEGF expression, ultimately improving angiogenesis. The results show that the topical injection of MSC improves wounds [37]. According to the results of a study, it is reported that MSCs accelerate the proliferation and migration of residual epithelial cells from deprived areas by the release of TGF-beta, EGF, FGF, and various inflammatory cytokines. In response to deep tissue injury, MSCs proliferate on epithelial cells to form a new basement membrane and then proliferate and migrate to repair all epithelial tissues.

Okamoto et al. have shown that the damaged epithelium is restored by bone marrow-derived cells in the human gastrointestinal tract. The results of one study showed that administration of MSCs, NO, or MSCs together with NO might have therapeutic effects on mucosal lesions in the gastric ulcer through anti-inflammatory, angiogenic and anti-apoptotic actions [38]. Although MSCs application did not significantly affect all the factors studied, it positively affected all. These cells improved the thickness of the stomach, increased anti-inflammatory factors, and the effect on mouse weight. It suggests that the use of cell therapy along with other methods can help accelerate the healing of gastric ulcers.

The use of herbal ingredients such as S. officinarum juice and G. glabra extract can be a therapeutic option for the treatment of gastric ulcer. These effects are probably due to the compounds present in these plants that require further studies to report more accurately.

5-Ethical Considerations

Authors approve that we protect animal rights in accordance with Guide for the Care and Use of Laboratay Animal and obtain an ethicl committee approval report in accordance of "Guide for the Care and Use of Laboratory Animals (1996)":

https://nap.nationalacademies.org/catalog/5140/guide-for-the-care-and-use-of laboratory-animals

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