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Original Research

Evaluation of Therapeutic Efficacy of Stigma and Petal Extracts of *Crocus sativus* L. on Acetic Acid-Induced Gastric Ulcer in Rats

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Abstract

Saffron (*Crocus sativus* L.) has long been considered a medicinal plant in Traditional Persian Medicine (TPM) due to its therapeutic properties. Despite this interest, its effects on gastrointestinal disorders have not been completely taken into consideration. Hence, this study aimed to evaluate the pharmacological activity of ethanolic extracts of saffron stigma (SS) and saffron petal (SP) in acetic acid-induced gastric ulcer in rats. The gastric ulcer model was imitated by the serosal application of acetic acid in male Wistar rats. Then, the animals were orally fed with 100 mg/kg and 200 mg/kg of ethanolic extracts of SS or SP, omeprazole (40 mg/kg), or saline for 12 days. The macroscopic and microscopic appearances of gastric ulcers and the levels of malondialdehyde (MDA), vascular endothelial growth factor (VEGF), and prostaglandin E2 (PGE2) in gastric tissues were assessed. The highest anti-ulcer activity was observed in the omeprazole-treated animals with the lowest ulcer size (4.29 ± 1.78 mm2). SS could not reduce gastric ulcer size in rats. Compared to the untreated rats, SP treatment significantly decreased ulcer indices in a dose-dependent manner. The gastric levels of PGE2, VEGF, and MDA were significantly elevated in the untreated animals with gastric ulcers compared to rats in the control group. The SS extract suppressed the elevated PGE2 and VEGF levels at both doses, while SP did not have a significant influence. Both SS and SP treatments significantly ameliorated MDA levels in rats with gastric ulcers. Omeprazole treatment enhanced the PGE2 level and suppressed MDA contents, but it did not influence the VEGF level. In conclusion, our findings demonstrated that the saffron stigma has no significant effects on the gastric ulcer healing process, while its petals accelerate the process. This discrepancy can be attributed to the difference in the main secondary metabolites between saffron stigma and petals.

Keywords: *Crocus sativus*; Saffron; Stomach ulcer; Prostaglandins; Vascular endothelial growth factor; Malondialdehyde

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Introduction

A gastric ulcer is a break in the gastric mucosa of the stomach that extends through the muscularis mucosa layer. Between 10-15% of the world's population suffers from peptic ulcers (gastric and duodenal ulcers) [1]. It is generated by a lack of balance between the gastric mucosal protective and destructive factors [2]. Gastric ulcer is usually caused by *Helicobacter pylori* (*H. pylori*) infection, chronic use of non-steroidal anti-inflammatory drugs (NSAIDs), smoking and alcohol consumption [3]. Currently, therapies aim to kill *H. pylori* bacterium, if present, and neutralize gastric acid, usually through proton pump inhibitors (PPIs) or medications to reduce acid production [4]. Although the global prevalence of gastric ulcers has been decreased, its management has become more challenging because the effectiveness of many regimens declined in recent years. Widespread use of NSAIDs, mainly among the elderly population (anti-thrombotic therapy), and an overall increase in the prevalence of *H. pylori* resistance to antibiotics are two leading causes of this challenge [5]. Numerous studies have indicated that the overuse of PPIs in benign conditions such as dyspepsia and gastroesophageal reflux disease has been increased over the past decades. Evidence shows an association between long-term PPIs use and increased risk of gastric neoplasia, hepatorenal damage, hypomagnesemia, bone fractures, and dementia [6,7].

Numerous clinical and experimental studies have demonstrated that herbal medicines could exhibit therapeutic benefits for gastric ulcers [8-10]. *Crocus sativus* L. or saffron (Iridaceae) is one of the most potent and famous drugs in Traditional Persian Medicine (TPM) prescribed for a variety of diseases [11]. Avicenna (the most famous physician in the TPM) in Book II, Canon of Medicine (al-Qanun fi al-tib), has mentioned a wide range of beneficial effects of saffron on gastrointestinal disorders. Avicenna stated that saffron could reduce the appetite by suppressing stomach acidity and strengthening the stomach and liver because of its warming, tonic, and astringent properties [12]. Razi, another famous physician in TPM, in his book, Al-Hawi, has noted that saffron neutralizes gastric acid, facilitates digestion of food, strengthens liver and stomach, and decreases appetite [13]. Based on the TPM literature, saffron has been traditionally used to treat depression, ocular disorders, asthmatic problems, palpitations, hepatic disorders, and gastrointestinal complications [14]. The evidence-based research has supported various medicinal properties of saffron, such as anti-diabetic, anti-cancer, cardioprotective, anti-depressant, and anti-bacterial [15,16]. More recently, saffron petals have attracted researchers' interest in investigating its pharmacological activities. A significant number of experimental studies demonstrated that saffron petals have many pharmacological features such as antioxidant, anti-nociceptive, anti-spasmodic, anti-bacterial, immunomodulatory, anti-depressant, anti-diabetic, hepatorenal protective, and anti-hypertensive activities [16-18]. The phytochemical analysis of saffron has shown that safranal, crocin, crocetin, and picrocrocin are the main characteristic compounds of the saffron stigma, while they are not present, except

for a few amounts of crocin (0.6%), in saffron petals. Three flavonols glycosides, namely kaempferol-3-O-sophoroside (62.19–99.48 mg/g), kaempferol-3-O-glucoside (27.74–45.18 mg/g), and quercetin-3-O-sophoroside (6.21–10.82 mg/g), have been detected as the important bioactive components of saffron petals [17,19].

As stated above, saffron has been prescribed for gastrointestinal disorders in the TPM. As far as we know, few studies have focused on the effects of saffron on gastrointestinal disorders like gastric ulcers. A limited number of studies have investigated the protective efficacy of saffron (or its active compounds, namely crocin and safranal) on indomethacin/various necrotizing agents–induced gastric damage in rats. They reported that saffron could prevent gastric lesions in animals by regulating oxidant-antioxidant balance and normalizing gastric pH [20-22].

Although gastric ulcers are usually chronic, most ulcer lesions induced by ethanol/indomethacin heal quickly in a few days without scar formation; hence, these models are often suitable for preventive studies [23]. Therefore, the present study aimed to evaluate the curative efficiency of ethanolic extracts of saffron stigma (SS) or saffron petal (SP) in acetic acid-induced gastric ulcers in rats for the first time.

Methods

Plant collection and extraction preparation

Saffron flowers were purchased from a local traditional market at Birjand, Iran. An expert botanist identified them, and a voucher specimen (H. No. 2669) was deposited in the herbarium of the faculty of agriculture at the University of Birjand, Iran. The petals and stigmas were separated and air-dried at room temperature. The dried plant parts were powdered by an electric grinder (Moulinex AR1043-UK) separately. The powders were macerated in 80% ethanol 1:10 (w/v) for 48 h at room temperature. Then, it was passed through filter papers (Blue Ribbon, Grade 589, Germany) and concentrated under a vacuum evaporator (Wiggens, Italy) at 45 °C. The resulting residue was transferred to 120 mm Petri dishes (10 mL per dish) and allowed to dry at 45 °C [24]. The yields (w/w) of SS and SP ethanolic extracts were 37.5% and 19%, respectively.

Animals

Adult male Wistar rats (200-220 g) were obtained from the Research Centre of Experimental Medicine, Birjand University of Medical Sciences, Birjand, Iran. All procedures involving animals were in accordance with the National Guide for the Care and Use of Laboratory Animals in Scientific Affairs provided by the Iranian Ministry of Health and Medical Education. In addition, the animal experiments were approved by the Birjand University of Medical Sciences ethics committee (IR.BUMS.1394.371). Rats were housed under standard conditions (22 ± 2 ºC, 30-35% humidity, and a 12-h light/dark cycle) and fed with the standard laboratory pelleted diet (Javaneh-Khorasan, Iran) and tap water. They were kept in propylene plastic cages with raised wire mesh floors to prevent coprophagia at the time of fasting.

The animals (n = 80) were randomly divided

into eight groups (Figure 1), each consisting of 10 rats: 1; control group (C), 2; sham-operated group (SO), 3; ulcer model group (MD), 4; omeprazole group (OM), 5; saffron stigma at the dose of 100 mg/kg (SS100), 6; saffron stigma at the dose of 200 mg/kg (SS200), 7; saffron petal at the dose of 100 mg/kg (SP100) and 8; saffron petal at the dose of 200 mg/kg (SP200). All mentioned allocations were made randomly after gastric ulcer induction or sham operation. Groups 1 to 3 were treated with saline (1 mL normal saline), and group 4 was treated with omeprazole 40 mg/kg (Dr. Abidi co., Iran) dissolved in the 0.9% normal saline [25]. All treatments were performed orally, once per day for 12 consecutive days. The doses of SS and SP, animal number per group, and the study period were carefully chosen based on previous studies

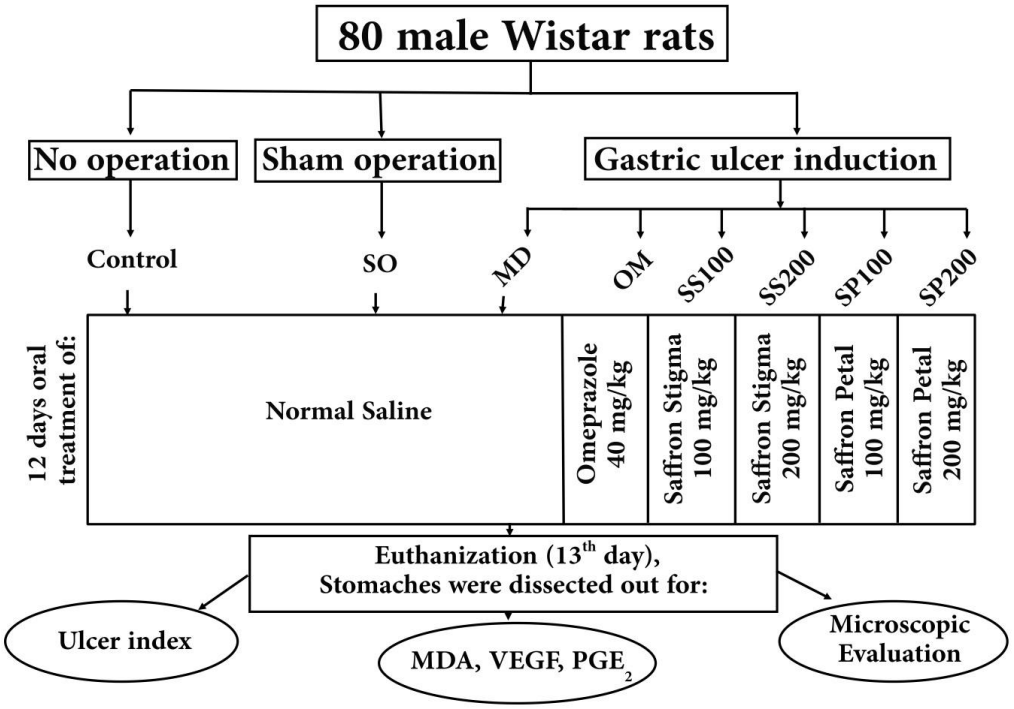

Figure 1. Schematic representation on the study design. SO: sham-operated group; MD: untreated gastric ulcer model group; OM omeprazole treated (40 mg/kg) group; SS100 and SS200: ethanolic extract of the saffron stigma at doses of 100 mg/kg and 200 mg/kg respectively; SP100 and SP200: ethanolic extract of the saffron petal at doses of 100 mg/kg and 200 mg/kg, respectively; MDA: malondialdehyde; VEGF: vascular endothelial growth factor; PGE2: prostaglandin E2.

Acetic acid-induced gastric ulcer model and drug administration

In order to test the anti-ulcer activity of SS and SP extracts, the acetic acid-induced gastric ulcer model was developed in rats. The model efficiently and reliably produces round (equal size) and deep ulcers in the stomach wall that highly resemble chronic human ulcers in gross appearance and histological aspects [29,30]. After 24 h of food deprivation, rats were anesthetized with intraperitoneal (i.p.) injections of ketamine and xylazine (80:10 mg/kg) [31]. Then, a laparotomy was performed through a midline incision, and the stomach was carefully exteriorized. After that, the serosal surface of the glandular portion of the stomach (5 mm away from pylorus)

was exposed to 120 µL of 40% acetic acid (Merck, Germany) for 90 s via a glass tube (9-mm internal diameter) tightly placed on it [32]. Afterward, acetic acid was removed using a surgical micro-suction, and the stomach was washed with saline; the abdomen was closed, and animals were received normal water and food supply. The same procedure was performed for sham-operated rats (n = 10) with normal saline instead of acetic acid. On the next day, the rats were grouped as described above and received their treatments. Their treatment lasted for 12 days. It is well demonstrated that gastric ulcer lesions induced by the serosal application of acetic acid became chronic within 2-3 days and eventually healed after 2-3 weeks [33]. Therefore, 12 days of the investigation were selected for the present study.

On the 13th day, animals were anesthetized with Ketamine/Xylazine solution (80:10 mg/kg), and their abdomen was opened. Their stomachs were dissected out, cut open along the greater curvature, and washed with ice-cold normal saline. The ulcer area was captured promptly and cut into two equal parts. One part was immediately fixed with 4% paraformaldehyde fixative solution for histological examination, and another part (around 100 mg) was used for biochemical assessment and kept at -70 ºC until analysis.

Assessment of stomach levels of VEGF, PGE2, and MDA

An equal weight of gastric tissue (100 mg) was homogenized in 900 µL phosphate-buffered saline (PBS) and centrifuged (3000 rpm) for 20 minutes at 4ºC [34]. Supernatants were collected and transferred to other tubes. Total protein content was quantified using a commercial protein assay kit (NS-15073, Navand Salamat, Iran).

The tissue contents of vascular endothelial growth factor (VEGF) and prostaglandin E2 (PGE2) were determined by commercially available ELISA kits (eBioscience, Austria for VEGF; Cayman Chemical Company, USA for PGE2).

The thiobarbituric acid reactive species (TBARS) method was used to determine the lipid peroxidation level. In this method, the malondialdehyde (MDA) level as the main end product of the lipid peroxidation process was calculated. In brief, 100 µL of the supernatant was added to 200 µL of 0.67% thiobarbituric acid, and 600 µL of 1% O-phosphoric acid, and the mixture was placed in a water bath (90 □) for 45 min. Then, samples were placed on the ice to stop the reaction, and 800 µL N-butanol was added to each sample, vortexed, and the butanol phase was separated by centrifugation at 5000 rpm for 20 min at 4 °C. The supernatant was collected (200 µL), and its absorbance was measured spectrophotometrically at 532 nm. Finally, the MDA level was expressed as nmol/g protein [35].

Macroscopic and microscopic analysis

The ulcer areas were measured with Image J software (1.44p; National Institute of Health, USA) and considered the ulcer index [36].

For microscopic evaluation, paraffin blocks of tissue samples were prepared by routine histological methods. In addition, sections (5 µm

thick) were prepared using a rotary microtome (Leitz 1512, Italy) and stained with hematoxylin and eosin dyes. Finally, the histological assessment was performed in at least three randomly selected slides from each rat under a light microscope (Euromex-CMEX-10, Netherland).

Statistical analysis

Data were analyzed using the statistical software IBM SPSS version 22. Values are shown as mean ± standard deviation (SD). The normality of data was checked using the Shapiro-Wilk test. Statistical comparisons were analyzed using one-way analysis of variance (ANOVA), and post hoc analysis was done using Tukey's test. P values less than 0.05 were considered significant.

Results

Macroscopic findings

The macroscopic appearance of stomachs in different studied groups is displayed in figuer 2. Meanwhile, no epithelial lesions were found in the C and SO groups, but a single circular ulcer with the central necrotic area (black area) was observed in the MD group.

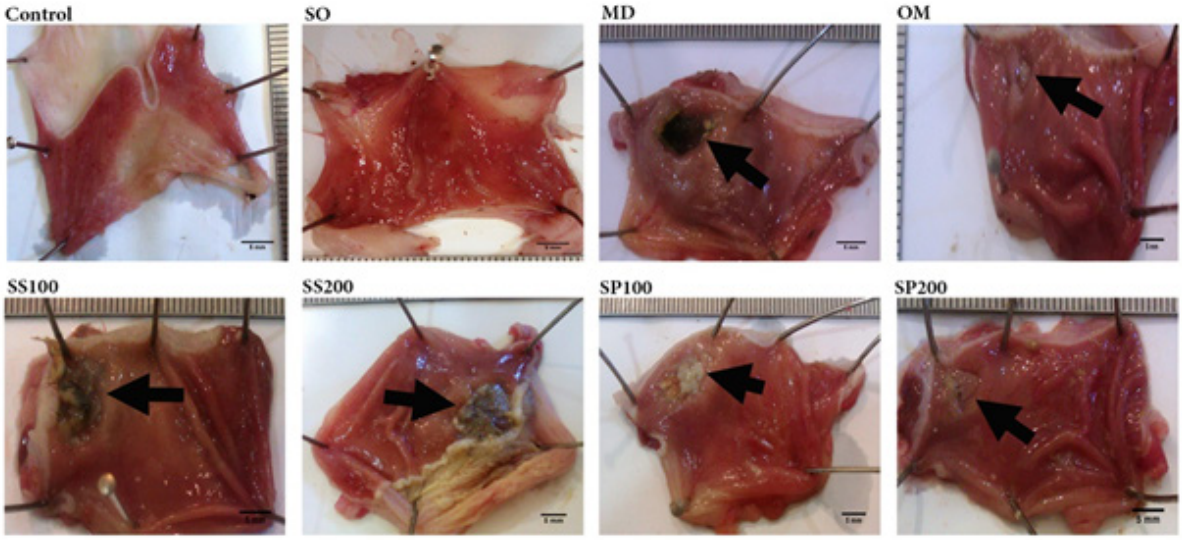

Figure 2. Macroscopic appearances of gastric mucosa belong to different studied groups. SO: sham-operated group; MD: untreated gastric ulcer model group; OM omeprazole treated (40 mg/kg) group; SS100 and SS200: gastric ulcer rats treated with ethanolic extract of the saffron stigma at doses of 100 mg/kg and 200 mg/kg respectively; SP100 and SP200: gastric ulcer rats treated with ethanolic extract of the saffron petal at doses of 100 mg/kg and 200 mg/kg, respectively. The black arrow indicates ulcer location. Scale bar = 5 mm.

The ulcer sizes of the studied groups were measured and considered as the ulcer indexes (Figure 3). The ulcer index of the MD group was 44.13 ± 9.18 mm2. Treatment with the SS extract at both doses could not significantly reduce ulcer indexes compared to the MD group (37.60 ± 3.30 mm2 in SS100 and 36.81 ± 8.04 mm2 in SS200). No significant difference was found between SS100 and SS200 groups concerning ulcer indexes. Treatment with the SP extract significantly reduced ulcer indexes in gastric ulcer rats in a dose-dependent manner. Compared to the MD group, the ulcer indexes of SP100 (32.36 ± 6.32 mm2; $p < 0.05$) and SP200 (20.01

± 3.61 mm2; $p < 0.01$) groups were significantly decreased. The ulcer index of the SP200 group was significantly lower than the SP100 group ($p < 0.01$). The highest healing efficiency was observed in the OM group with a 4.29 ± 1.78 mm2 ulcer index which was lower than all experimental groups ($p < 0.001$).

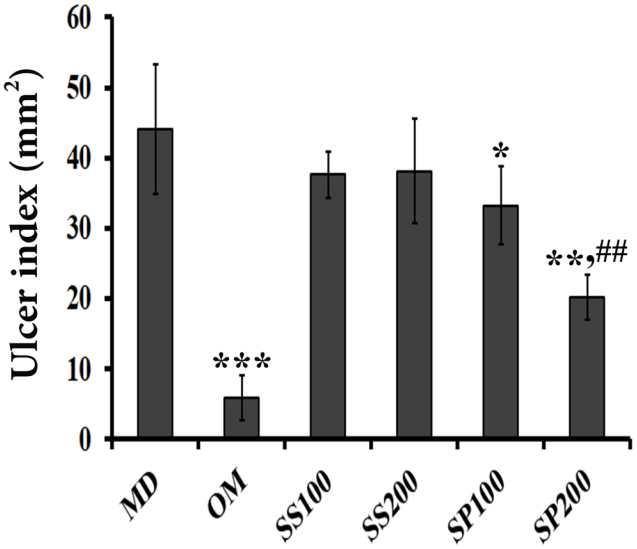

Figure. 3. Ulcer index (mm2) of untreated gastric ulcer model (MD), 40 mg/kg omeprazole treated (OM), saffron stigma treated at doses of 100 mg/kg (SS100) and 200mg/kg (SS200) and saffron petal treated at doses of 100 mg/kg (SP100) and 200 mg/kg (SP200) groups. *, **, *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ significant differences compared to the MD group, respectively. ## shows significant difference ($p < 0.01$) compared to the SP100 group.

Microscopic findings

In agreement with macroscopic findings, histological evaluation confirmed the presence of an ulcer penetrating the entire gastric wall in the MD group (Figure 4).

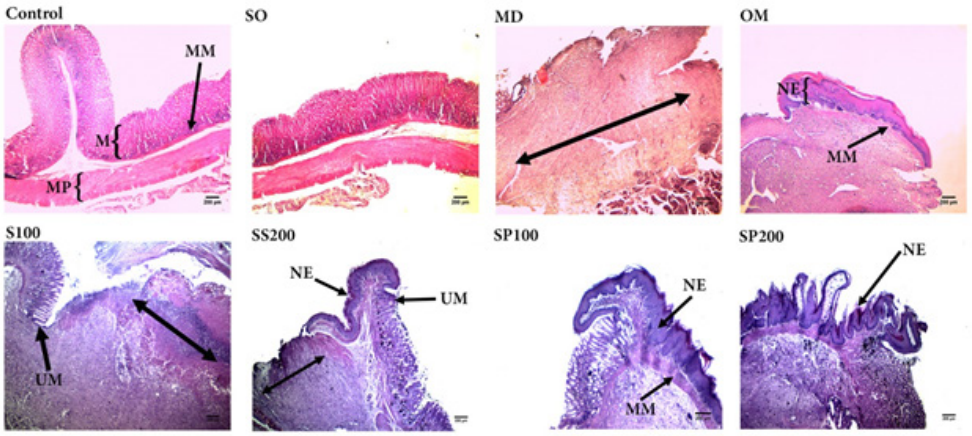

Figure 4. Stomach histopathology in different groups. SO: sham-operated group; MD: untreated gastric ulcer model group; OM: omeprazole (40mg/kg) treated groups; SS100 and SS200: saffron stigma (100 and 200 mg/kg) treated groups; SP100 and SP200: saffron petal (100 mg/kg and 200 mg/kg) treated rats. The control and SO groups show normal histology of the stomach with normal structures of mucosa (M), muscularis mucosa (MM), and muscularis propria (MP). In the MD and SS groups, a large fibrotic area (double head arrows) is observed between ulcer margins (UM). Formation of the new epithelium (NE) and MM are observed in the OM and SP groups. Incomplete re-epithelialization is evident in the SS200 group. Hematoxylin and eosin staining, 40X magnification, scale bar =200 µm

Microscopic examination of the SO group revealed typical structures of glandular epithelium and gastric mucosa. In contrast, in the MD group, the mucosal epithelium was absent in the ulcer area, and many degenerative changes, including leucocytes exudate, inflammatory cells infiltration, and mucosal hemorrhage around ulcer margins, were evident (ulcer development phase). However, the gastric tissue of the OM group revealed the healing phase with the apparent re-epithelialization accompanied by muscularis mucosa reconstruction. In the SS100 group, superficial mucosal erosions remained present and, in the SS200 group, incomplete re-epithelialization of the ulcer area was observed. Histological assessments also indicated that treatment with the SP extract promoted healing of the gastric ulcer by forming new epithelium and mucosa regeneration (SP200) and reducing inflammatory cell infiltration (SP100).

Effects on VEGF levels

The results of stomach VEGF levels in the different studied groups are shown in figure 5A. There was no statistical difference between the C and SO groups (92.06 ± 5.87 pg/mL vs. 95.14 ± 5.90 pg/mL; $p = 0.99$). The VEGF levels increased significantly in the MD (133.38 ± 18.88 pg/mL; $p < 0.01$) and OM (142.71 ± 11.44; $p < 0.001$) groups compared to the SO group. Compared to the MD group, the SS treatment at both doses could significantly decrease the VEGF levels ($p < 0.01$ for both). No significant difference was found between the SS100 (93.76 ± 6.36 pg/mL) and SS200 (96.66 ± 6.53 pg/mL) groups. On the other hand, treatment with the SP extract at both doses did not influence VEGF level in gastric ulcer rats compared to the MD group. Also, no statistical difference was found between the SP100 and SP200 groups considering VEGF levels (118.85 ± 12.22 pg/mL vs. 122.40 ± 4.97 pg/mL; $p = 0.99$).

Effects on PGE2 levels

The gastric ulcer induction caused a significant increase in the stomach level of PGE2 (168.60 ± 25.65 ng/L) when compared to the SO (60.30 ± 6.41ng/L; $p < 0.001$) and C (60.03 ± 4.45ng/L; $p < 0.001$) groups (Figure. 5B). The omeprazole treatment elevated the PGE2 level even more than the MD group (209.00 ± 30.74 ng/L; $p = 0.004$). In contrast, the SS administration at both doses markedly suppressed PGE2 levels (114.33 ± 8.59ng/L in SS100 and 115.50 ± 14.30ng/L in SS200) in rats' gastric ulcer ($p < 0.001$). The PGE2 levels in the SP100 (140.42 ± 18.89 ng/L) and SP200 (134.40 ± 16.87 ng/L) groups were not significantly different compared to the MD group.

Effects on MDA levels

As shown in figure 5C, the MDA level of the MD group was statistically higher than the SO group (10.80 ± 0.86 nmol/g vs. 8.98 ± 0.61 nmol/g, $p < 0.001$). Thus, all treatments could significantly ameliorate MDA elevation in gastric ulcers of animals. On the other hand, there was no statistical difference in MDA levels comparing OM, SS, and SP groups.

Discussion

In the present study, the ulcer healing activity of

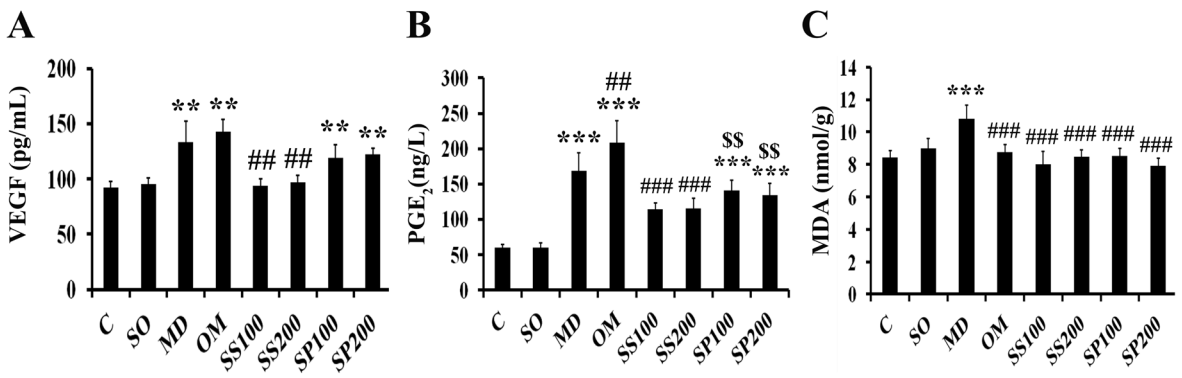

Figure. 5. Gastric contents of vascular endothelial growth factor (VEGF) (A), prostaglandin E2 (PGE2) (B) and malondialdehyde (MDA) (C) of rats in control (C), sham-operated (SO), omeprazole (OM), saffron stigma extract-treated at doses of 100-200 mg/kg (SS100, SS200) and saffron petal extract-treated at doses of 100-200 mg/kg (SP100, SP200) groups. Values are presented as mean± SD. (n= 10). ** and *** indicate $p < 0.01$ and $p < 0.001$ significant differences compared to the C group, respectively. ## and ### show $p < 0.01$ and $p < 0.001$significant differences compared to the MD group. $$ indicates a $p < 0.01$ significant difference compared to the OM group.

the SS and SP ethanolic extracts was evaluated on acetic acid-induced gastric ulcers in rats in a 12-day study. Moreover, three probable mechanisms involving the ulcer healing process were evaluated by measuring tissue VEGF, PGE2, and MDA contents. Our findings revealed that the SS could not exhibit valuable ulcer healing efficiency; whereas, the SP showed gastric ulcer healing activity, particularly in the maximum dose (200 mg/kg). Furthermore, although the SS extract suppressed VEGF and PGE2 levels, the SP extract did not influence these parameters. On the other hand, both SS and SP could efficiently prevent lipid peroxidation by decreasing MDA levels.

Generally, ulcer healing is an orchestrated process in which mucosal defects fill within the connective tissue and epithelial cells. Typically, a gastric ulcer consists of an ulcer margin within non-necrotic mucosa, and a granulation lesion consists of connective tissue at the ulcer base [37]. The healing process of gastric ulcers involves different stages, including cell proliferation and differentiation, cell migration, extracellular matrix deposition, active angiogenesis, and reconstruction [38]. Epithelial cells from the ulcer margin proliferate and migrate onto the granulation tissue, re-epithelialize the ulcer and finally reconstruct the glands [37]. Therefore, this process is controlled by different cellular mediators, including growth factors, transcription factors, and cytokines. Among these mediators, prostaglandins and VEGF play crucial roles in regulating gastric acid secretion and the acceleration of ulcer healing [39]. PGE2 is a vital factor in epithelial cell proliferation by the modulation in signaling pathways, including PI3K/Akt and the Wnt cascade [37,39,40]. In the present experiment, omeprazole remarkably enhanced the PGE2 level. In contrast, in the SS-treated rats, the gastric levels of PGE2 were decreased significantly compared to the untreated rats. Several studies have confirmed the anti-inflammatory effects of saffron stigma. Besides, the anti-inflammatory activity of saffron stigma can be attributed to its colored carotenoids, crocin. Xu and colleagues investigated some pharmacological activities of crocin

and reported that it could inhibit the production of cyclooxygenase 1 and 2 enzymes.

Moreover, they found that crocin inhibited the release of PGE2 in lipopolysaccharide-challenged macrophage cells in a dose-dependent manner [41]. The SS extract also decreased the VEGF levels in rats' gastric ulcers. It is speculated that the SS extract reduced the VEGF levels through two pathways. There is evidence showing that PGE2 can stimulate VEGF expression through several mechanisms like up-regulating CXCR4 (SDF-1 receptor) and activation of the hypoxia-inducible factor-1α (HIF-1α) [42,43]. Accordingly, the SS extract probably inhibited VEGF expression by suppressing the PGE2 production in the gastric tissue.

Moreover, numerous studies have indicated the anti-angiogenic potential of saffron stigma as an underlying mechanism of its anti-cancer activity [44]. Besides, evidence has demonstrated that crocetin, the aglycone of crocin, suppressed VEGF-induced tube formation in human umbilical vein endothelial cells by inhibiting p38 phosphorylation [45]. This finding indicates that the anti-angiogenic activity of saffron stigma is not limited to cancer conditions. The other important metabolite of saffron stigma is safranal which is responsible for the saffron aroma. More recently, Tammadonifard et al. studied the gastro-protective effects of safranal against indomethacin-induced gastric lesions in rats. They have pre-treated rats by i.p. administration of safranal (0.063, 0.25, and 1 mg/kg) for seven days, and after that, gastric lesions were induced by oral administration of indomethacin (50 mg/kg). Six hours after the indomethacin administration, the animals were sacrificed, and their gastric contents were investigated [22]. They found that safranal normalized gastric volume and pH and prevented gastric lesions formation in a dose-dependent manner. These results do not support our findings. There are several differences between the mentioned study and our investigation, which can justify these inconsistencies. In the present study, a chronic gastric ulcer model was established, and the therapeutic potential of SS/SP extracts was investigated, while in the study of Tammadonifard et al., a gastric lesion model for studying the gastroprotective potential of safranal was used. The indomethacin-induced gastric lesion model is used to ascertain whether the stimulation of cyclooxygenase activity mediates the anti-ulcer (not ulcer healing) properties of a substance/agent or not. In the acetic acid-induced gastric ulcer model, the mucosal damage reached the mucous membrane and submucosa and the muscles (like chronic human gastric ulcer) [46]. In addition, in the mentioned study, safranal was administrated through the i.p. route, whereas, in our study, oral administration of the extracts (SS/SP) was performed. From the pharmacokinetic point of view, the route of administration can influence the bioaccessibility, bioavailability, and bioactivity of saffron's compounds [47]. Furthermore, Nabavizadeh et al. reported that five-day oral pretreatment with the aqueous extract of saffron increased the basal and stimulated acid/pepsin output by increasing nitric oxide [48].

On the other hand, the SP extract could not effectively influence the PGE2 and VEGF levels.

Consequently, more proliferation and repair were observed in the SP-treated gastric ulcer rats than in the SS-treated animals. To the best of our knowledge, no study investigated the protective or anti-ulcer activity of saffron petals on gastric ulcers. As mentioned before, the saffron petal is considered a rich source of kaempferol. Besides, Li and colleagues investigated the protective effect of kaempferol (40-160 mg/kg) against acute ethanol-induced lesions to the gastric mucosa in mice. They reported that kaempferol decreases pro-inflammatory cytokines levels and improves nitric oxide production through inhibiting neutrophil accumulation [49]. Thus, it seems that the anti-inflammatory effect of saffron petals is not due to their effect on cyclooxygenase enzymes and prostaglandins.

It is well established that reactive oxygen species (ROS) play an essential role in the progression of various degenerative disorders like gastric ulcers. In the current study, the MDA level elevation in ulcerative animals due to failure in antioxidant defense mechanisms was observed. The present study results confirmed the antioxidant activities of saffron stigma/petals by decreasing and normalizing the MDA levels. In agreement with our findings, different studies have found that saffron stigma or petal could ameliorate serum or tissue MDA level in different stressful conditions such as diabetes, toxicity, ischemia, and peptic lesions [26,34,50].

The current study has potential limitations. So, this study's first and foremost limitation is using non-standardized extracts that could be addressed in future research.

Conclusion

In conclusion, the present study findings demonstrated that saffron stigma has a no significant effects on the gastric ulcer healing process. It is probably due to the presence of crocin in the saffron stigma. Nevertheless, the saffron petal extract exhibited ulcer healing activity in acetic acid gastric ulcers in rats through antioxidant and anti-inflammatory effects without affecting the PGE2 pathway.

Competing Interests

All authors declare that they have no competing interest.

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