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Safranal, a constituent of saffron, exerts gastro-protective effects against indomethacin-induced gastric ulcer

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carboxymethylcellulose sodium (Pubchem CID: 6328154)
tiobarbituric acid (Pubchem CID: 2723628)
sodium dodecyl sulphate (Pubchem CID: 3423265)
acetic acid (Pubchem CID: 176)
2,4,6-tripyridyl-8-$FeCl_3$·$6H_2O$ (Pubchem CID: 6093258)
Tween 80 (Pubchem: 5281955)

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ABSTRACT

Aims: Several natural products have been evaluated for management of gastric ulcer induced by non-steroidal anti-inflammatory drugs. Safranal, a plant-derived chemical, has a potent antioxidant and anti-inflammatory properties. The present study was aimed to evaluate possible gastro-protective effects of safranal against indomethacin-induced gastric ulcer in rats. Lansoprazole (a proton pump inhibitor) was used as a reference drug.
Materials and methods: Thirty rats were divided into five groups. Groups 1 and 2 received vehicle. Groups 3, 4 and 5 treated with 0.063, 0.25 and 1 mg/kg safranal. Group 6 received 30 mg/kg lansoprazole. All groups except of group 1 received indomethacin (50 mg/kg) ingestion. Six hours later, animals were euthanized and their stomachs were removed. Gastric contents volume and pH were measured. Gastric ulcer area and protective index were evaluated using image J software. Histological changes were evaluated by light microscope. Malondialdehyde (MDA) level, superoxide dismutase (SOD) activity, total antioxidant capacity (TAC) content, tumor necrosis factor-alpha (TNF-α) and Caspase-3 levels were determined in the gastric tissue.
Key findings: Safranal and lansoprazole normalized gastric volume and pH, reduced gastric ulcer area and produced gastric protection. Indomethacin-induced histological changes and tissue biochemical alterations were ameliorated by the above-mentioned treatments.
Significance: The results of the present study suggest the involvement of anti-secretory, anti-oxidant, anti-inflammatory and anti-apoptotic mechanisms in gastro-protective effect of safranal. In addition, gastro-protective effect of safranal was comparable to lansoprazole.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, aspirin and diclophenac are widely used for their analgesic, anti-inflammatory and antipyretic properties [1]. These drugs produce undesired side effects on gastric mucosa such as gastric mucosal damage [2]. This side effect is very common and may evolve in gastric ulcer leading to serious complications such as perforation and bleeding, if not treated adequately [3]. Inhibition of acid secretion using proton pump inhibitors (PPIs), histamine H_2 receptor blockers, eradication of *Helicobacter pylori* (*H. pylori*) with antibiotics and therapeutic regimens are the common gastric ulcer therapies [4]. These treatments are effective, but their side effects limit clinical utility [5]. In this context, preclinical and clinical studies have demonstrated that medicinal plants and their active substances exhibit therapeutic benefits for gastric ulcer with fewer side effects [6].

Saffron (*Crocus sativus* L.) has been used in traditional medicine for treating several diseases such as depression, asthma and insomnia, and these beneficial effects are due to major constituents of the plant such as crocetin, crocins and safranal [7]. Safranal ($C_{10}H_{14}O$), as the most abundant chemical in saffron essential oil, is responsible for odor of this plant [8]. This apocarotenoid, safranal, exerts tissue protective effects due to potent anti-oxidant, anti-inflammatory and anti-apoptotic properties [9,10].

The PPIs including omeprazole, esomeprazole, lansoprazole, dexlansoprazole, pantoprazole and rabeprazole are a class of pharmaceutical agents that suppress gastric acid secretion by H^+/K^+ ATPase inhibition [11]. These drugs are widely used in the treatment of gastric acid related diseases such as peptic ulcer disorders [12]. However, PPIs long-term use may lead to potential side effects such as osteoporotic

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fracture, renal damage, dementia, vitamin B_{12} and iron deficiencies and anemia [13].

Saffron and its constituents, especially crocin, exert potential therapeutic properties against different digestive system disorders such as peptic ulcer, stomach and pancreas cancers and ulcerative colitis [14]. Only in one study, a preventive effect of acute oral administration of safranal against indomethacin-induced gastric tissue changes of malondialdehyde (MDA) level and glutathione activity has been found in non-diabetic and diabetic rats [15]. In the present study, gastro-protective effects of safranal were investigated on indomethacin-induced gastric ulcer by physiological (gastric contents volume and pH and ulcer area), histopathological (light microscopy) and biochemical (oxidative stress, inflammation and apoptosis) evaluations. We also used lansoprazole as a reference drug to compare the obtained results.

2. Materials and methods

2.1. Animals

In the present study, healthy adult male Wistar rats (220–250 g) were used. Animals were maintained in an animal house of physiology laboratory under controlled conditions (ambient temperature 22 ± 0.5 °C and 12 h light-dark cycles) with food and water *ad libitum*. Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University approved the study protocol.

2.2. Chemicals

Safranal liquid (purity of ≥88%), lansoprazole, indomethacin and carboxymethyl cellulose (CMC—Na) were purchased from Sigma-Aldrich (Sigma-Aldrich, Chemical Co., St. Louis, MO, USA). Analytical chemicals such as sodium dodecyl sulfate, acetic acid, thiobarbituric acid, 2,4,6-tripyridyl-*S*-triazine (TPTZ), *n*-butanol, pyridine and $FeCl_3{\cdot}6H_2O$ were purchased from Merck Chemical Co., Darmstadt, Germany.

2.3. Experimental groups

Thirty-six rats were divided into six groups of six rats in each group, as following:

Group 1 (Vehicle + Vehicle): This group received safranal and indomethacin vehicles vehicle by intraperitoneal injection and gavage administration, respectively.

Group 2 (Vehicle + Indo 50): This group received safranal vehicle and indomethacin.

Groups 3 (Saf 0.063 + Indo 50), 4 (Saf 0.25 + Iindo 50) and 5 (Saf 1 + Indo 50): In these groups, after administration of safranal at doses of 0.063, 0.25 and 1 mg/kg, respectively, indomethacin was ingested.

Group 6 (Lpz 30 + Indo 50): This group received indomethacin ingestion after an oral administration of 30 mg/kg lansoprazole.

To explore the effects of chemicals in rats without induction of gastric ulcer, 24 rats were divided into four groups with six rats in each group for administration of safranal (0.063, 0.25 and 1 mg/kg) and lansoprazole (30 mg/kg) for seven consecutive days. In these groups, in addition to collection of acid secretion, macroscopic and microscopic and biochemical changes evaluations on day seven, initial (day 1 before chemical administration) and final (day 7) recordings of body weight, fecal output and general behavior (grooming and locomotor activities) were performed.

2.4. Treatment schedule

Safranal liquid and lansoprazole were dissolved in normal saline with adding two drops Tween 10%. Safranal at doses of 0.063, 0.25 and 1 mg/kg was intraperitoneally injected at a constant volume of 1 ml/kg [9]. Lansoprazole at a dose of 30 mg/kg was administered by gavage [16]. Safranal and lansoprazole were administered for seven consecutive days before induction of gastric ulcer.

2.5. Induction of gastric ulcer

Gastric ulceration was induced by indomethacin as described previously [16]. On day seven of treatments, animals were deprived of food but had free access to water for 24 h before ulcer induction. Indomethacin was suspended in CMC-Na and administered at a dose of 50 mg/kg by intra-gastric tube to all groups except of group one (vehicle + vehicle group).

2.6. Gastric content volume and pH measurement

Six hours after indomethacin ingestion, under ketamine (100 mg/kg) and xylazine (10 mg/kg) deep anesthesia, the abdomen was opened and gastric pylorus was ligated and the stomach was removed and opened through large curvature. Immediately, the entire gastric content was collected in centrifuge tubes. These tubes were centrifuged at 2500*g* for 5 min to remove any solid debris and the volumes of supernatant were measured. Gastric contents pH was determined using a digital pH meter (PHscan 40, Pocket PH tester, BANTE, China).

2.7. Gastric ulcer area measurement

After removing of gastric contents, mucosa was rinsed with cold normal saline and blotted dry and stretched on a piece of filter paper with the mucosal surface facing up and photographed with a digital camera and stored in a computer. In the photographed stomachs, the ulcer area (mm^2) was measured using Image J software (Image J 1.46r, Wayne Rasband, National Institute of Health, USA). The protective index was calculated using the following formula: [(Ulcer $area_{(indomethacin)}$-Ulcer $area_{(treated)}$)/Ulcer $area_{(indomethacin)}$] × 100 [17].

2.8. Gastric tissue sampling

Each stomach was dichotomized; one moiety was placed in 10% formalin solution for histopathologic assessment while the other moiety was stored at −80 °C for biochemical determination.

2.9. Microscopic evaluation

Formalin-fixed stomachs routinely processed for paraffin embedding, thin (4–5 μm) sections were provided, and stained with hematoxylin and eosin (H&E) for light microscopic observations. From the histological slides, histopathological changes including congestion, hemorrhages, inflammatory cell infiltration, edema and sloughing were scored using a 0–3 scale (0 = none; 1 = mild; 2 = moderate; and 3 = sever changes) as previously described [18].

2.10. Biochemical assay

Biochemical assay was made on stomach tissue homogenates. The stomach tissue segments were cut into small pieces and homogenized at 4 °C in 3 ml of ice-cold saline using a glass homogenizer.

2.10.1. Evaluation of gastric tissue MDA

Gastric tissue MDA level was measured by the thiobarbituric acid (TBA) method as described previously [19]. Peroxidation was measured as the production of MDA, which in combination with TBA forms a pink chromogen compound whose absorbance was measured spectrophotometrically (JASCO, UV–975, Tokyo, Japan) at 532 nm. Gastric tissue MDA level was expressed as nmol/mg protein.

2.10.2. Evaluation of gastric tissue SOD

Gastric tissue activity of SOD was determined by Superoxide

Dismutase Assay Kit (Item No.706002) according to the manufacture instruction (Cayman Chemical, 1180E. Ellsworth Rd. Ann Arbor, MI, USA). Gastric tissue SOD activity was expressed as U/mg protein.

2.10.3. Evaluation of gastric tissue TAC

Gastric tissue activity of TAC was determined by measuring the ability to reduce Fe^{3+} to Fe^{2+} (FRAP: ferric-reducing antioxidant capacity power) as described previously [20]. The reagent included 2,4,6-tripyridy-*S*-triazine (TPTZ), $FeCl_3$ and acetate buffer. The complex between Fe^{2+} and TPTZ gives a blue color with absorbance at 593 nm. Gastric tissue TCA activity was expressed as mmol/mg protein.

2.10.4. Evaluation of gastric tissue TNF-α

The level of TNF-α in the stomach tissue homogenates was measured by ELISA assay according to the kit instruction (Rat TNF-α, Platinum ELISA, affymetrix eBioscience, North America). Gastric tissue TNF-α level was expressed as pg/mg protein.

2.10.5. Evaluation of gastric tissue caspase-3

Gastric tissue Caspase-3 level was determined using ELISA assay according to the kit instruction (ELISAKIT, Elabscience Biotechnology Co., Ltd., www.elabscience.com). The level of Caspase-3 was expressed as ng/mg protein.

2.10.6. Evaluation of gastric tissue protein

Protein concentration of stomach tissue was measured using Bradford protein assay [21].

2.11. Statistical analysis

Statistical comparisons were performed using the GraphPad Prism (Version 5) software (GraphPad software, San Diego, CA, USA). The results obtained from stomach contents volume and pH, ulcer area and biochemical changes were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Because of semi-quantitative nature of data obtained from microscopic scoring, Kruskal-Wallis and *post hoc* Nunn tests were applied. The score values were presented as quartiles minimum value, first quartile, median, third quartiles and maximum value and the other data were expressed as the mean ± SEM. Significance at $p < 0.05$ has been given receptive in all tests.

3. Results

3.1. Effects of safranal and lansoprazole on some experimental parameters in rats without induction of gastric ulcer

Seven days treatments with safranal and lansoprazole did not alter body weight, fecal output and general behaviors. No significant changes in physiological, pathological and biochemical parameters were observed among safranal, lansoprazole and vehicle plus vehicle treated groups (data not shown).

3.2. Effects of safranal and lansoprazole on gastric content volume and pH

Fig. 1 shows the effects of safranal and lansoprazole on gastric contents volume (A) and pH (B) changes induced by indomethacin. In vehicle + vehicle group, gastric content volume and pH were 1.03 ± 0.09 ml and 4.41 ± 0.29 unit, respectively. Vehicle + indomethacin group showed a significant ($p < 0.001$, Fig. A) increase in gastric contents volume (2.84 ± 0.19 ml) and a significant ($p < 0.001$, Fig. B) decrease in gastric contents pH (1.95 ± 0.16 unit). The increased gastric content volume and the decreased gastric content pH were not significantly ($P > 0.05$) changed by 0.063 mg/kg safranal. Safranal at doses of 0.25 mg/kg ($p < 0.01$) and 1 mg/kg ($p < 0.001$) significantly decreased gastric content volume (Fig. 1A) and increased gastric content pH (Fig. 1B). Lansoprazole (30 mg/kg) significantly ($p < 0.001$) decreased gastric content volume and increased gastric content pH (Fig. 1A and B). Lansoprazole(30 mg/kg) and safranal (1 mg/kg) produced similar significant ($p < 0.001$) effects (Fig. 1A and B).

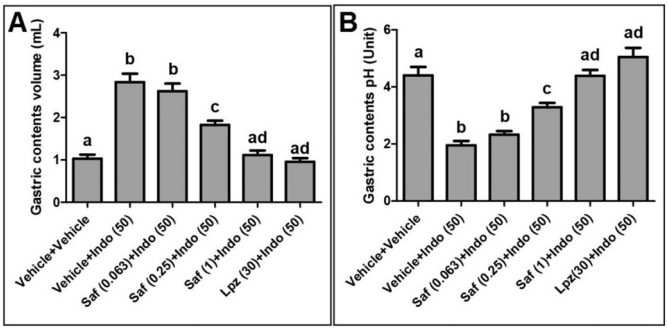

Fig. 1. Effects of safranal and lansoprazole on gastric contents volume (A) and pH (B) changes induced by indometacin in rats. Data are presented as Mean ± S.E.M. ($n = 6$ rats in each group). Similar letters denote no significant differences (a *vs* a: $p > 0.05$ and b *vs* b: $p > 0.05$). Non-similar letters indicate significant differences between groups (a *vs* b: $p < 0.001$, a *vs* c, b *vs* c and c *vs* d: $p < 0.05$ and b *vs* d: $p < 0.001$). The numbers inside the parenthesis indicate the chemical doses used in mg/kg body weight. Indo; indomethacin, Saf: safranal, Lpz: lansoprazole.

3.3. Effects of safranal and lansoprazole on ulcer area and protective index

In vehicle + vehicle group, gastric mucosa showed normal architecture (Fig. 2A), whereas extensive gastric ulcers were dominant in vehicle + indomethacin group (Fig. 2B). Safranal at a dose of 0.063 mg/kg did not alter (Fig. 2C), whereas at doses of 0.25 and 1 mg/kg it reduced gastric ulcer severity (Fig. 2D and E). Lansoprazole at a dose of 30 mg/kg (Fig. 2F) reduced gastric ulceration severity. Ulcer area (Fig. 2G) and protective index (Fig. 2H) were 0.00 ± 0.00 mm^2 and 100 ± 0.00%, respectively, in vehicle + vehicle treated group. These values significantly ($p < 0.001$) reached to 6.59 ± 0.99 mm^2 (Fig. 2F) and 0.00 ± 0.00% (Fig. 2G) in vehicle + indomethacin treated group. Safranal at a dose of 0.063 mg/kg did not change, whereas at doses of 0.25 mg/kg ($p < 0.01$) and 1 mg/kg ($p < 0.001$) it significantly decreased the increased ulcer area and increased the decreased protective index induced by indomethacin (Fig. 2G and H). Lansoprazole (30 mg/kg) and safranal (1 mg/kg) produced similar significant ($p < 0.001$) effects in reducing ulcer area, while lansoprazole-elevated protective index was significantly ($p < 0.05$) more than safranal (Fig. 2H).

3.4. Effects of safranal and lansoprazole on histological changes of gastric mucosa

Gastric mucosa of vehicle + vehicle-treated group had normal architecture (Fig. 3A) with 0.00 ± 0.00 histopathological median scores (Fig. 3F–J). Histopathological changes including congestion, inflammatory cell infiltration, edema and sloughing (Fig. 3B1, 3B2 and 3B3) were observed and median scores of histopathological changes were 2.83 (congestion, Fig. 3G), 2.83 (inflammatory cell infiltration, Fig. 3H), 2.75 (edema, Fig. 3I) 2.92 (sloughing, Fig. 3J). Safranal at a dose of 0.063 mg/kg was without effect (Fig. 3C and Fig. 3G–J), whereas at doses of 0.25 and 1 mg/kg it significantly ($P < 0.05$) reduced gastric mucosa histopathological (Fig. 3D and F) as well as score (Fig. 3G–J) changes induced by indomethacin. Lansoprazole at a dose of 30 mg/kg significantly ($p < 0.05$) decreased indomethacin-induced histopathological (Fig. 3F) and score (Fig. 3G-3J) changes in gastric mucosa. Suppressive effects of safranal (0.25 and 1 mg/kg) and lansoprazole (30 mg/kg) on gastric mucosal lesion were similar.

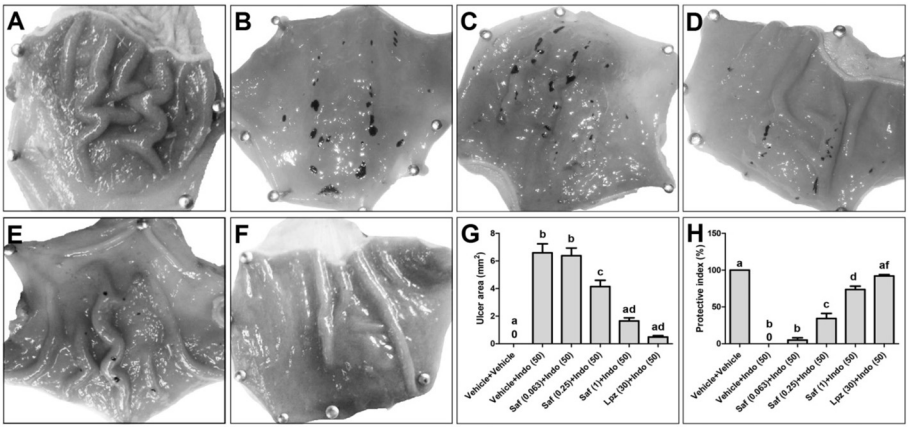

Fig. 2. Gross appearance of the stomachs. (A) Vehicl + vehicle group shows normal gastic mucosa. (B) Vehicle + indomethacin (50 mg/kg) group shows extensive gastric mucosal lesions. (C) Safranal (0.063 mg/kg) + indomethacin (50 mg/kg) group shows extensive gastric mucosal lesions. (D) Safranal (0.25 mg/kg) + indomethacin (50 mg/kg) group shows moderate lesion severity in gastric mucosa (E) Safranal (1 mg/kg) + indomethacin (50 mg/kg) treated group shows minimal lesion severity in gastric mucosa. (F) Lansoprazole (30 mg/kg) + indomethacin (50 mg/kg) group shows no gastric mucosal ulcer. (G) Ulcer area (mm^2) and protective index (H) data are presented as mean ± SEM. Similar letters denote no significant differences (a *vs* a: $p > 0.05$, b *vs* b: $p > 0.05$). Non-similar letters indicate significant differences among groups (a *vs* b: $p < 0.001$, b *vs* c: $p < 0.05$, b *vs* d: $p < 0.001$and b *vs* f: $p < 0.001$, c *vs* f: 0.01 and d *vs* f: $p < 0.05$). The numbers inside the parenthesis indicate the chemical doses used in mg/kg body weight. Indo; indomethacin, Saf: safranal, Lpz: lansoprazole.

3.5. Effects of safranal and lansoprazole on gastric tissue MDA, SOD and TAC

Gastric tissue MDA level (Fig. 4A), SOD (Fig. 4B) and TAC (Fig. 4C) activities were 5.48 ± 0.32 nmol/mg protein, 120.07 ± 4.69 U mg/protein and 0.66 ± 0.04 mmol mg/protein, respectively, in vehicle + vehicle group. These biochemical parameters significantly ($P < 0.001$) reached to 16.67 ± 0.82 nmol mg/protein (MDA), 31.58 ± 2.68 U mg/protein (SOD) and 0.26 ± 0.02 mmol mg/protein-1 (TAC) in indomethacin treated group. Safranal at a dose of 0.063 mg/kg produced non-significant ($p > 0.05$) effects (Fig. 4A–C), whereas at a dose of 0.25 mg/kg it significantly ($p < 0.01$) decreased the increased level of MDA (Fig. 4A) and increased the decreased activities of SOD (Fig. 4B) and TAC (Fig. 4C). Safranal (1 mg/kg) and lansoprazole (30 mg/kg) treatments showed similar significant ($p < 0.001$) reducing effects on MDA level (Fig. 4A) and elevating effects on SOD (Fig. 4B) and TAC (Fig. 4C) activities.

3.6. Effects of safranal and lansoprazole on gastric tissue TNF-α and caspase-3

In vehicle + vehicle group, gastric tissue levels of TNF-α (Fig. 5A) and caspase-3 (Fig. 5B) were 42.2 ± 4.64 pg/mg protein and 13.47 ± 1.26 ng/mg protein, respectively. Vehicle + indomethacin treated group showed significant ($p < 0.001$) elevations in TNF- α (103.93 ± 3.53 pg/mg protein) and caspase-3 (34.55 ± 1.61 ng/mg protein) levels of gastric mucosa. Safranal at a dose of 0.063 mg/kg produced non-significant ($p > 0.05$) effects (Fig. 5A and5B), whereas at a dose of 0.25 mg/kg it significantly ($p < 0.01$) decreased the increased levels of TNF-α (Fig. 5A) as well as caspase-3 (Fig. 5B) induced by indomethacin in gastric tissue. Safranal (1 mg/kg) and lansoprazole (30 mg/kg) produced similar significant ($p < 0.001$) reducing effects on the increased TNF-α (Fig. 5A) and caspase-3 (Fig. 5B) levels.

4. Discussion

The results of the present study showed that indomethacin produced

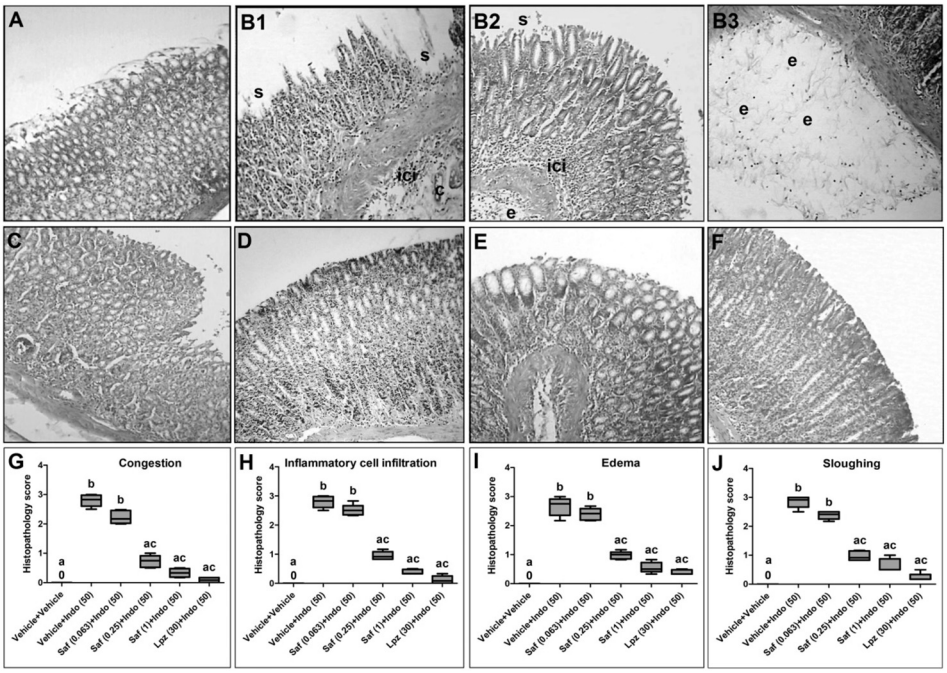

Fig. 3. Light transverse micrographs of the stomachs. (A) Vehicle + vehicle group shows normal mucosa and submucosa. (B1–B3) Vehicle + indomethacin (50 mg/kg) group shows congestion (c), inflammatory cell infiltration (ici), edema (e) and sloughing (s). (C) Safranal (0.063 mg/kg) + indomethacin (50 mg/kg) shows a very weak recovery of mucosa and submucosa. (D) Safranal (0.25) + indomethacin (50 mg/kg) shows a moderate recovery of mucosal epithelium and submucosal edema. (E) Safranal (1 mg/kg) + indomethacin (50 mg/kg) and (F) lansoprazole (30 mg/kg) + indomethacin (50 mg/kg) show a strong recovery of mucosa and submucosa structures. (H& E × 100). Histological scores of congestion (G), inflammatory cell infiltration (H), edema (I) and sloughing (J) are presented as quartiles: minimum value, first quartile, median, third quartiles and maximum value. (n = 6 rats in each group). Similar letters denote no significant differences (a *vs* a: $p > 0.05$, b *vs* b and c *vs* c: $p > 0.05$). Non-similar letters indicate significant differences among groups (a *vs* b: $p < 0.01$ and b *vs* c: $p < 0.05$). The numbers inside the parenthesis indicate the chemical doses used in mg/kg body weight. Indo; indomethacin, Saf: safranal, Lpz: lansoprazole.

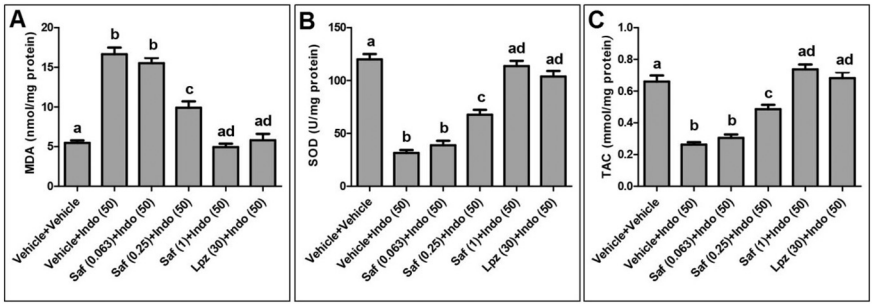

Fig. 4. Effects of safranal and lansoprazole on MDA (A) level and SOD (B) and TAC (C) activity changes of gastric tissue induced by indomethacin in rats. Data are presented as Mean ± S.E.M. (n = 6 rats in each group). Similar letters denote no significant differences (a *vs* a: $p > 0.05$ and b *vs* b: $p > 0.05$). Non-similar letters indicate significant differences among groups (a *vs* b: $p < 0.001$, a *vs* c, b *vs* c and c *vs* d: $p < 0.05$ and b *vs* d: $p < 0.001$). The numbers inside the parenthesis indicate the chemical doses used in mg/kg body weight. Indo; indomethacin, Saf: safranal, Lpz: lansoprazole.

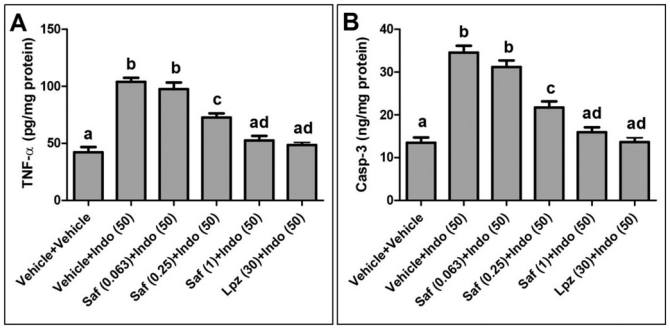

Fig. 5. Effects of safranal and lansoprazole on TNF-α (A) and caspase-3 (B) level changes of gastric tissue induced by indometacin in rats. Data are presented as Mean ± S.E.M. (n = 6 rats in each group). Similar letters denote no significant differences (a *vs* a: $p > 0.05$ and b *vs* b: $p > 0.05$). Non-similar letters indicate significant differences among groups (a *vs* b: $p < 0.001$, a *vs* c, b *vs* c and c *vs* d: $p < 0.05$ and b *vs* d: $p < 0.001$). The numbers inside the parenthesis indicate the chemical doses used in mg/kg body weight. Indo; indomethacin, Saf: safranal, Lpz: lansoprazole.

gastric ulcer by alteration of gastric secretion, induction of histopathological changes in gastric mucosa and elevation of gastric tissue MDA, TNF-α, caspase-3 levels and suppression of SOD and TAC activities. Indomethacin-induced gastric ulcer in rats is frequently used as a standard model to study pathophysiological mechanisms and pharmacological intervention of gastric ulcer [16,17]. The important mechanisms involved in the pathogenesis of indomethacin-induced gastric ulcer are inhibition the releasing of protective factors such as prostaglandin E_2, bicarbonate and mucus, misbalancing oxidative and anti-oxidative status, overproduction of inflammatory and apoptotic biomarkers and microvasvular disturbances and inflammatory cell infiltration due to induction of gastric hypermotility [22–24].

Our present results indicated that both safranal and lansoprazole produced similar improving effects on gastric content volume and pH changes induced by indomethacin. Gastric content volume and pH reflects gastric acid secretion induced by parietal cell H+/K+ ATPase activity which is under neurocrine (acetylcholine), endocrine (gastrin), paracrine (histamine), and intracellular (cAMP-and calcium-signaling pathways) regulation [11]. In addition to inhibitory effect on serum histamine elevation in ovalbumin sensitized guinea pigs [25], safranal affects cell membrane proteins such as activation of β_2-adrenoceptor, inhibition of histamine H_1 and muscarinic receptors, calcium channels as well as membrane bound proteins such as F1F0 ATP syntahse and α2R283D mutant ATP synthase [26,27]. In addition, safranal and its some semi-synthetic derivatives such as thiosemicarbazonic derivatives and the (thiazol-2-yl)hydrazonic compound have been found to exhibit potent anti-Helicobacter pylori activities [28]. Moreover, a protective effect of N-095, a saffron containing drug, has been reported against histamine-induced gastric ulcer in rats [29]. It has been reported that lansoprazole produced a potent anti-secretory effect on indomethacin-induced gastric secretion in rats [30]. In this context, omeprazole, another PPIs, increased pH value above the intact group in indomethacin-induced gastric ulcer [31].

Only in one study, the gastric ulcer index was reduced after administration of saffron extract, crocin and safranal 30 min before indomethacin (40 mg/kg) ingestion [15]. In this context, crocin and pantaprazole, reduced gastric index (mm^2) induced by indomethacin in rats [32]. Our present results also showed that safranal decreased ulcer area and increased protective index. Ulcer area, ulcer index and protective index have been frequently used to investigate the effects of anti-ulcer drugs, medicinal plants and their active constituents on macroscopic changes of gastric mucosa induced by indomethacin [31–36].

In the present study, safranal and lansoprazole alleviated indomethacin-induced histopathological changes including congestion, inflammatory cell infiltration, edema and sloughing in the gastric mucosa. Although ameliorating effects of crocin on indomethacin-induced gastric histopathological changes have been reported previously [32], there are no reports showing the effects of safranal on gastrointestinal mucosa changes induced by NSAIDs. However, in other experimentally-induced pathological states, safranal produced ameliorating effects on histopathological changes [25,37]. Local epithelial damage, lymphocyte infiltration, bleeding and lung congestion were ameliorated by safranal in ovalbumin sensitized guinea pigs [25]. In addition, Wallerian degeneration and edema of sciatic nerve tissue were improved by safranal in STZ-induced type 1 diabetic rats [37]. In the hepatic injury induced by intrarenal aortic occlusion, safranal alleviated histological changes such as dilation, congestion and inflammatory cell infiltration in sinusoidal area [38]. In experimentally-induced gastric ulcer, lansoprazole (30 mg/kg) produced improving effects on gastric mucosal histopatological changes such as epithelial cell loss, hemorrhage, inflammatory cell infiltration and edema [16,17].

Oxidative and anti-oxidative imbalance is a well-known mechanism involving in NSAIDs-induced gastric ulcer [24]. MDA is one of the products of lipid peroxidation and is the most frequently measured biomarker of oxidative stress status in cells and tissues injury [39]. On the other hand, SOD reflects the protection of cells and tissues against toxic effects of superoxide radicals and TAC is most frequently used to determination of free-radical antioxidant balance in biological systems [40,41]. Our present study results showed that the increased level of MDA and the decreased activities of SOD and TAC induced by indomethacin have been similarly normalized by safranal and lansoprazole. This is in agreement with the other findings in which similar improving effects of safranal and omeprazole have been reported against indomethacin-induced gastric tissue changes of MDA level and glutathione (GSH) activity [15]. In addition, oral administration of lansoprazole (30 mg/kg) for seven consecutive days prevented indomethacin-induced elevation of MDA level as well as reduction of SOD and GSH activities in the gastric tissue [16].

In the resent study, the increased levels of TNF-α in the gastric tissue that induced by indomethacin were similarly decreased by safranal and lansoprazole. TNF-α, as one of the most aggressive factors in the inflammation and injury, reduces microcirculation around the ulcer and delays healing due to potentiation of inflammatory response [42]. There are no reports showing the effects of safranal on gastric tissue

level in gastric ulcer. However, in other experimentally-induced pathological conditions, safranal produced reducing effect on tissue level of TNF-α. For example, safranal recovered diabetes-induced memory impairment by reducing TNF-α level of hippocampal tissue [9]. In addition, safranal treatment suppressed immunoreactivity and expression of the inflammatory cytokines IL-1β and TNF-α in a rat model of traumatic injury to the spinal cord [43]. Lansoprazole (30 mg/kg) treatment markedly decreased the increased gastric tissue TNF-α level and expression in indomethacin-induced gastric ulcer [16,44].

In addition to ant-secretory, anti-oxidative and anti-inflammatory effects, we also investigated the anti-apoptotic effects of safranal and lansoprazole against indomethacin-induced gastric ulcer by gastric tissue level measurement of caspase-3. Our results showed that safranal and lansoprazole produced similar reducing effects on the increased caspase-3. Caspases are a family of cysteine proteases whose functions are inextricably linked with the process of inflammation (caspase-1, -4, -5, -11 and -12) and apoptosis (caspase-2, -3, -7, -8, -9 and -10) [45]. In this context, caspase-3 expression increases after induction of gastric ulcer by ethanol and NSAIDs [46,47]. There are no reports showing anti-apoptotic effect of safranal in various models of experimentally induced gastric ulcer. In a rat model of spinal cord injury, TUNEL staining and electron microscopy revealed that safranal treatment inhibited injury-induced apoptosis [43]. In addition, the increased level of caspase-3 in the hippocampus was improved by safranal treatment in type-1 diabetic rats [9]. Moreover, apoptotic detectors such as Cyt c, survivin, p44/42 MAPK (ERK1/2), Phospho-p44/42 MAPK (ERK1/2), PI3 Kinase P85, Phospho-PI3 Kinase P85, phospho SAPK/JNK, SAPK/JNK and caspase-3 that induced by PC12 cell exposure to β-amyloid and hydrogen peroxide were inhibited by pre-exposing to safranal [48]. On the other hand, lansoprazole increased gastric tissue mRNA expression of COX-1 and COX-2 enzymes and prostaglandin E_2 level and reduced TUNEL staining apoptosis and Bax expression in indomethacin-induced gastric ulcer model in rats [17].

In our present study, safranal and lansoprazole produced similar gastro-protective effects against indomethacin-induced gastric ulcer. Medicinal plant extracts, their biologically active constituents and PPIs have been frequently used to compare anti-ulcer activities. For example, berberine and coptisine, active constituents of *Copits cheinensis*, and lansoprazole produced approximately similar anti-ulcer effects aginst indomethacin-induced gastric ulcer [16]. In addition, pogostone (high dose), one of the major constituents of *Pogostemonis herba*, and lansoprazole produced similar stimulatory effects on cyclooxygenase-mediated prostaglandin E_2 production, anti-oxidative and anti-apoptotic mechanisms in indomethacin-induced gastric ulcer [17]. In this context, omeprazole produced 87.73%, 85.5% and 86.12% and *Salmalia malaborica extract* showed 74.54%, 78.14% and 78.84% protective effects against aspirin-, alcohol-, and pylorus ligation models of gastric ulcer, respectively [49]. Although proton pump inhibitors are the most widely used therapeutic agents in NSAIDs-induced gastric ulcer, their serious adverse effects such as chronic kidney disease, cognitive decline, myocardial infarction, stroke, bone fracture and even death can limit clinical use [50]. Our present results showed no adverse effects of safranal (0.063, 0.25 and 1 mg/kg) during seven days treatment protocol. In this context, no toxic effects have been reported after long-term (5 weeks) use of 0.2 and 0.8 mg/kg safranal [9]. However, a low toxic effect of safranal by reducung blood hematocrit, hemoglobin and platelets has been reported after long-term (21 days) oral administration of this apocarotenoid in rats [51]. Human and animal models studies have demonstrated that medicinal plants with fewer adverse effects but comparable and even superior effects than drugs such as PPIs and histamine H_2 receptor blockers can produce more beneficial effects against gastric ulcer [6,52].

In conclusion, the results of the present study showed that indomethacin produced gastric ulceration by stimulation of acid secretion, activation of oxidative stress, suppression of antioxidant system, overproduction of inflammatory and apoptotic biomarkers. Safrana, a constituent of saffron, and lansoprazole (a PPI drug) exerted similar gastro-protective effects by anti-secretory, anti-oxidant, anti-inflammatory and anti-apoptotic mechanisms.

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Disclosures

The authors declare that there are no competing interests.

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