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Research Article
Icariin Attenuates Indomethacin-Induced Gastric Ulcer in Rats: Emphasis on Antioxidant, Anti-Inflammatory and Pro-Angiogenic Properties

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Abstract

Background and Objective: A gastrointestinal tract disorder called a gastric ulcer is typified by the deterioration of the stomach's protective lining. Over time, the non-selective NSAID indomethacin (INDO) that is widely used damages the gastric lining, resulting in the development of gastric ulcers. A flavonol glycoside, Icariin (ICA), has certain pharmacological characteristics, including anti-inflammatory and antioxidant qualities. The present research aimed to examine the protective properties of ICA on rats against stomach ulcers caused by INDO. **Materials and Methods:** The 5 groups of rats were established. As the Control group: 0.5% sodium carboxymethylcellulose (1 mL per 100 g). INDO group was administered indomethacin at a dosage of 50 mg/kg, the OMEP 30 group received omeprazole at 30 mg/kg, the ICA 25 group was given 25 mg/kg of Icariin, and the ICA 50 group received 50 mg/kg of Icariin, all for a duration of seven days, with the exception of the INDO group. Every group, except the control, received INDO (50 mg/kg) 1 hr after their treatments on the seventh day. Ulcer index, histopathological changes, oxidative stress and inflammatory and angiogenic markers were assessed. Statistical analysis was carried out by one-way ANOVA followed by Tukey's test. **Results:** The ICA significantly decreased the ulcer index and ameliorated histopathological alterations induced by INDO. Further, ICA exhibited anti-ulcer effect by enhancing the antioxidant enzyme activities, cyclooxygenase-1 (COX-1) and prostaglandin E2 (PGE2) levels, as well as anti-inflammatory activities by attenuating the rise in Interleukin-6 (IL-6), Tumor Necrosis Factor-Alpha (TNF-α), Interleukin-1β (IL-1β) and Nuclear Factor Kappa B (NF-κB) concentrations. The ICA also enhanced Vascular Endothelial Growth Factor (VEGF) levels in gastric tissues. **Conclusion:** The antioxidant, anti-inflammatory and angiogenic properties of ICA are responsible for its gastroprotective effects.

Key words: Gastric ulcer, indomethacin, icariin, oxidative stress, anti-inflammatory effect, angiogenesis property

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A gastric ulcer is a gastrointestinal tract disorder that involves the deterioration of the mucosal integrity of the stomach tissue, ranging from the muscularis mucosa to the submucosa or beyond[1]. The primary factors that promote gastric ulcers include *Helicobacter pylori*, stress, age and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)[2,3].

Globally, NSAIDs are frequently prescribed drugs and many users report experiencing gastrointestinal problems[4]. Non-selective NSAIDs like indomethacin (INDO) are frequently used to treat inflammatory diseases including arthritis[5]. The integrity of the stomach mucosa is altered by frequent use of INDO and this is a major contributor to the development of ulcers therein[6]. Additionally, the acidic properties of NSAIDs and their capacity to hinder the production of prostaglandin E2 (PGE2) by non-specifically inhibiting cyclooxygenases also contribute to mucosal damage and the emergence of gastric ulcers[7]. Other mechanisms comprise the release of free radicals, the generation of inflammatory mediators, the inhibition of angiogenesis and the induction of apoptosis[8-11].

A wide range of drugs (H2 antagonists, antimicrobials, proton pump inhibitors and antacids) can be used to prevent and treat stomach ulcers. Nevertheless, these drugs experience undesirable side effects and are ineffective in preventing the recurrence of ulcers[12-14]. The increasing prevalence of stomach ulcers has necessitated the development of novel gastroprotective substances. Regarding this matter, natural compounds with desirable pharmacological properties have been suggested due to their efficacy, safety and widespread public acceptability[15-17]. Earlier study indicates that medicines possessing antioxidant, anti-inflammatory, angiogenic and antiapoptotic properties aid in the prevention of stomach ulcers produced by indomethacin[18,19].

Epimedii has an extensive record of use in traditional Chinese medicine; it is also referred to as Yin Yang Huo in Chinese[20]. Icariin (ICA) is the primary biologically active substance found in Herba Epimedii. It possesses a diverse array of medicinal effects[21] including neuroprotection[22], cardiovascular protection[23], anti-cancer properties[24], immune protection[25] and enhancement of reproductive function[26]. Studies have shown that icariin and its metabolites have several protective benefits. These effects include enhancing the body's reaction to inflammation and oxidative stress, controlling the proliferation and apoptosis of cells and preventing damage to the vascular endothelial cells[23,27]. Nevertheless, there is a lack of evidence addressing its effectiveness in preventing indomethacin-induced stomach damage. Hence, this study intended to evaluate ICA's ability to reduce indomethacin-induced stomach ulcers in rats.

MATERIALS AND METHODS

Study area: The current study was conducted between October, 2023 and May, 2024 at the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia.

Drugs and chemicals: Omeprazole (OMEP), INDO, sodium carboxymethylcellulose (Na-CMC) and ICA (99% purity) were procured from Sigma Aldrich, St. Louis, Missouri, USA. The phosphate buffer, formaldehyde and other necessary supplies were of analytical grade.

Animals: The 30 male Wistar rats weighing between 200-220 g were collected from the vivarium of the Faculty of Pharmacy (FOP) at King Abdulaziz University (KAU), Jeddah, Saudi Arabia. The rats were kept in an accredited facility with 50-55% humidity and a temperature of 22±2°C. They were also exposed to a light/dark cycle of 12 hrs of light followed by 12 hrs of darkness. The study methodologies were examined by the Ethics Committee of FOP and KAU and approved (Reference number is PH-1444-51).

Experimental protocol: The 5 separate groups were made for the rats (n = 6). Control group: 0.5% sodium carboxymethylcellulose (1 mL per 100 g). INDO group was administered indomethacin at a dosage of 50 mg/kg, the OMEP 30 group received omeprazole at 30 mg/kg, the ICA 25 group was given 25 mg/kg of Icariin, and the ICA 50 group received 50 mg/kg of Icariin, all for a duration of seven days, with the exception of the INDO group. Following an initial trial, icariin dosages administered to the rats were established and they aligned with the findings from the prior investigation[28]. On the 6th day, the rats were subjected to 24 hrs of food deprivation while still being allowed unrestricted access to water. On the 7th day, all groups except the control group received INDO at a dosage of 50 mg/kg, 1 hr after their respective treatments. All the drugs were suspended in 0.5% Na-CMC and administered by oral gavage.

The animals were euthanized by exsanguination under isoflurane anesthesia, 6 hrs after INDO ingestion and the stomachs were isolated for experimental protocols.

Morphological and histological analysis: The stomachs were promptly extracted and cut across the larger curvature. Next, the entire stomachs were rinsed with sterile ice-cold saline solution. After that, pictures of rat stomachs were captured by

using a digital camera (Nikon, Japan). A macroscopic examination of the stomach's glandular mucosa was conducted to ascertain the existence of bleeding stripes. The length of every injury were quantified in millimeters (mm). The ulcer index (UI) was calculated following the method depicted by Sathish *et al.* [29]:

$$UI = \frac{10}{x}$$

Where:
x = Total mucosal area/total ulcerated area

The stomach samples were soaked in neutral buffered formalin (10%) for a whole day. Afterward, subjected to dehydration using a series of ethanol solutions of increasing concentration and later embedded in paraffin. Next, stomach slices of 4.0 µm thick were stained with Hematoxylin and Eosin (H&E). The aberrant alterations were analysed by a qualified histopathologist. The subsequent grading scheme was applied: (1) Inflammatory cell infiltration (score: 0-2), (2) Haemorrhage (score: 0-4), (3) Epithelial cell loss (score: 0-3) and (4) Mucosal erosions (score: 0-4)[30].

Tissue homogenate preparation: The gastric tissues were homogenized using a 10% ice-cooled phosphate-buffered saline solution (pH 7.4). The homogenates underwent a 20 min centrifugation at 10,000 g, 4°C. The supernatant was collected for further biochemical analyses.

Evaluation of oxidative stress indices: Using commercial kits and adhering to the manufacturer's instructions, the level of malondialdehyde (MDA) and the activities of catalase (CAT) and superoxide dismutase (SOD) were determined. (Cat. no.: Bio Diagnostic, Giza, Egypt; MD-2529, GR-2511, SD-2521 and CA-2517).

Assessment of COX-1 and PGE2: Rat COX-1 (Cat. # MBS703362 MyBioSource, Inc., San Diego, California, USA) and Rat PGE2 ELISA kits (cat # CSB-E07967r, Cusabio, Wuhan, China) were used to measure PGE2 activity in stomach tissue homogenates. This kit uses an enzyme immunoassay approach based on competitive inhibition.

Assessment of inflammatory markers: Stomach inflammation was evaluated by measuring the concentrations of IL-6, TNF-α, IL-1 β and total NF-κB p65 in the stomach tissue homogenate. The IL-6, TNF-α and IL-1 β levels were measured using commercial kits (cat # MBS726707, MBS282960 and MBS825017 MyBioSource, San Diego, CA, USA). The NF-κB p65 levels have been determined by commercially available kits (cat # CSB-E08788r, Cusabio, Wuhan, China).

Assessment of angiogenic marker: The gastric VEGF level was determined in the stomach tissue homogenate by employing a commercially available ELISA kit (cat #CSB-E04757r, Cusabio, Houston, Texas, USA).

Statistical analysis: The data are expressed as Means±Standard Deviations (SD). To assess significance, a One-way Analysis of Variance (ANOVA) was conducted to compare the variables across different experimental groups. Subsequently, Tukey's *post hoc* test was employed to identify specific differences among groups. For nonparametric histopathological scoring data, the Kruskal-Wallis test was utilized, followed by Dunn's test for further analysis. A significance level was set at $p<0.05$.

RESULTS

Effect of ICA on INDO-induced morphological and histological alterations: Gastric tissues from the control group presented normal structural patterns when examined macroscopically. There was no incidence of lesions or redness on the stomach mucosa of the rats in control (Fig. 1a). However, rats treated with INDO displayed hemorrhagic streaks on the surface of the gastric mucosa (Fig. 1b). Redness or impairment was absent following pretreatment with OMEP (Fig. 1c). Rats pretreated with ICA (25) flashed scattered bleeding strips, albeit to a substantially lesser extent than the animals subjected to INDO (Fig 1d). In contrast, those pretreated with ICA (50) showed minimum damage with intact mucosa (Fig. 1e). The data in Fig. 1f indicate the ulcer index. Administration of ICA (25 and 50) led to a noteworthy decrease in ulcer index by 63.32 and 91%, respectively, in contrast to the INDO group.

As shown in Fig. 2a, the control animals showed a regular histopathological appearance of stomach tissue. No damaged mucosa with intact parietal cells, submucosa and muscularis layer was seen. The control group showed no evidence of neutrophilic infiltration, inflammation, cellular disintegration or fibrotic changes. On the contrary, the INDO-treated group (Fig. 2b) showed marked cellular disintegration, damaged layers of mucosa and mucosal necrosis. Notably, superficial mucosa is severely damaged/disintegrated, with damaged submucosa, vacuolated cells and inflammatory cells along

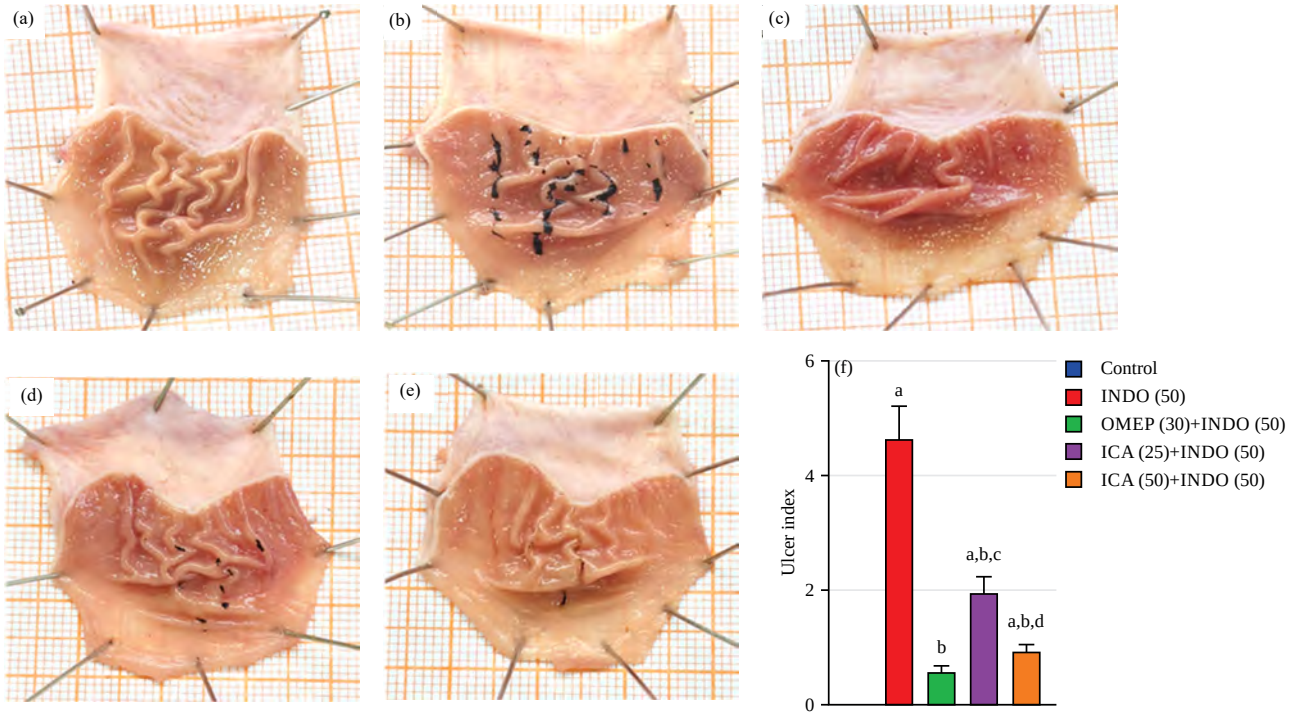

Fig.1(a-f): Photographs showing the rat's stomach tissue at a macroscopic level, (a) Control rats exhibit healthy mucus membrane, (b) INDO-treated rats showing hemorrhagic and ulcerated gastric mucosa, (c) OMEP (30) pretreated group showing no signs of injury or redness, (d) ICA (25) pretreated group exhibiting minor lesions, (e) ICA (50) pretreated group confirmed tiny injury with normal mucosa and (f) Graphical illustration of ulcer index
a, b, c, and d are substantially ($p<0.05$) dissimilar from control, INDO, OMEP, and ICA (25)+INDO (50), respectively

with fibrotic changes. When the animals were treated with OMEP and ICA (25 or 50), a marked reversal of histopathological aberrations caused by INDO, was seen in the case of OMEP (Fig. 2c). No marked improvement was seen in the case of the ICA (25) (Fig. 2d). On the contrary, pretreatment with ICA (50) showed moderate restoration of mucosal and superficial mucosa. Additionally, mild vacuolation and fibrotic changes, intact submucosa and muscularis layer were seen with the ICA (50) pretreatment (Fig. 2e) (stomach; H&E; 50 µm).

The evaluation of the histological anomalies disclosed by ICA (25 and 50) considerably averted the increase in the histological abnormalities in the stomach tissue produced by INDO (Fig. 2f).

Effect of ICA on oxidative stress markers: The results presented in Table 1 demonstrate that INDO markedly increased the amount of lipid peroxidation, as evidenced by the rise in the concentration of stomach MDA. Depletion of SOD and CAT activity followed this. By contrast, the animals pretreated with ICA (25 and 50) showed significant inhibition of MDA accumulation by 33.10 and 45.41%, SOD lowered activity by 43.0 and 91.5% and CAT exhaustion by 55 and 133%, respectively. Additionally, OMEP nearly completely prevented the oxidative stress caused by INDO, with MDA, SOD and CAT levels that were similar to those seen in control rats.

Effect of ICA on COX-1 activity and PGE2 levels: According to the findings in Fig. 3a, the concentration of COX-1 in the stomach tissue significantly decreased after exposure to INDO, by 78.57%, as contrasted to the control. Concerning the INDO, prior administration of ICA (25 and 50) markedly increased COX-1 levels (92.0 and 263%, respectively). In addition, compared to the INDO, pretreatment with OMEP showed a significant increase in COX-1 content (316%). The Fig. 3b shows a notable decrease (60.74%) in the level of PGE2 compared to the control group after INDO. In contrast to the INDO group, OMEP, ICA at (25 and 50) caused a significant increase in the PGE2 level, with increases of 128.70, 43.53 and 92.15%, respectively (Fig. 3b).

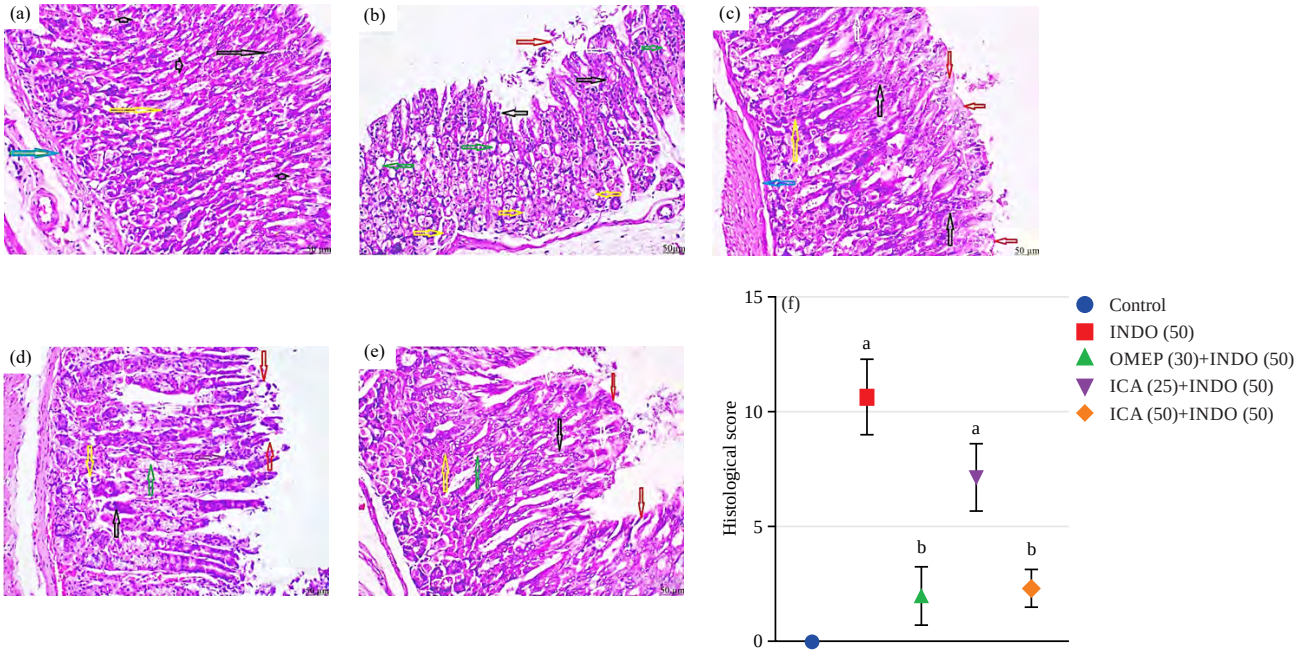

Fig. 2(a-f): Impact of ICA on histopathological modifications in stomach tissue caused by INDO, (a) Control group exhibited a typical histological structure of stomach tissue, (b) INDO-treated group showed marked cellular disintegration, (c) Pretreatment with OMEP showed a marked reversal of histopathological aberrations caused by INDO, (d) ICA (25) pretreated group showed no marked improvement, (e) ICA (50) pretreated group showed moderate restoration of mucosal and superficial mucosa (red arrow) and (f) An illustration of the histopathological score

Collected data were statistically analyzed by applying the Kruskal-Wallis test pursued by Dunn's test ($p<0.05$); [a,b]Substantially ($p<0.05$) dissimilar from control and INDO, respectively

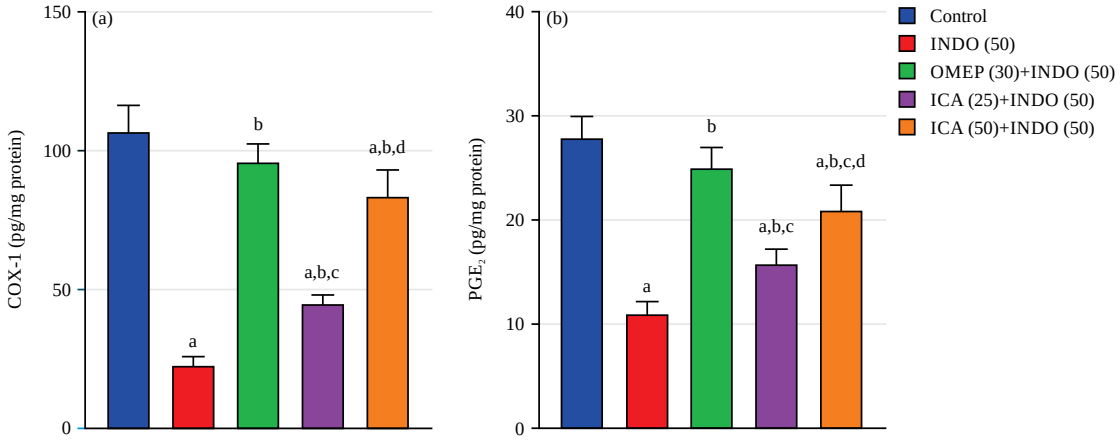

Fig. 3(a-b): Impact of ICA on (a) COX-1 and (b) PGE2

Stated values are Mean±SD (n = 6), [a,b,c,d]Substantially ($p<0.05$) dissimilar from control, INDO, OMEP and ICA (25)+INDO (50), respectively

Table 1: Impact of ICA on oxidative distress produced by INDO

	Control	INDO (50)	OMEP (30)+INDO (50)	ICA (25)+INDO (50)	ICA (50)+INDO (50)
MDA (nmol/mg protein)	1.693±0.22	3.49±0.38[a]	1.76±0.21[b]	2.33±0.23[a,b,c]	1.90+0.18[b,d]
SOD (U/mg protein)	9.41±0.81	4.36±0.75[a]	8.29±0.76[b]	5.80±0.74[a,b,c]	7.79±0.96[a,b,d]
CAT (U/mg protein)	0.311±0.022	0.106±0.020[a]	0.276±0.024[b]	0.165±0.018[a,b,c]	0.248±0.022[a,b,d]

MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, INDO (50): Indomethacin 50 mg/kg, OMEP (30): Omeprazole 30 mg/kg, ICA (25): Icariin 25 mg/kg, ICA (50): Icariin 50 mg/kg, values are displayed as Mean±SD (n = 6), [a,b,c,d]Substantially ($p<0.05$) dissimilar from control, INDO, OMEP and ICA (25)+INDO (50), respectively

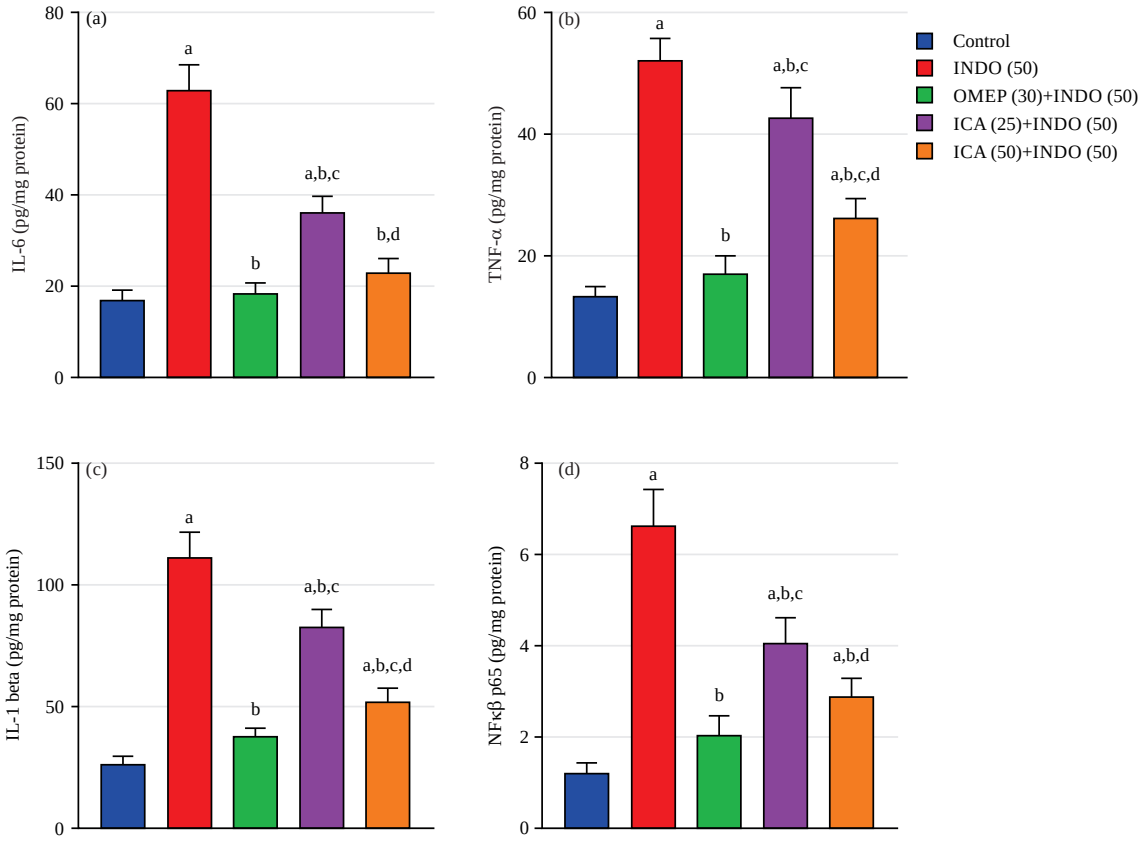

Fig. 4 (a-d): Impact of ICA on indicators of inflammation; (a) IL-6, (b) TNF-α, (c) IL-1β, and (d) Total NF-κβ-p65 in gastric tissue

The Information is displayed as Mean±SD (n = 6), [a,b,c,d]Substantially ($p<0.05$) dissimilar from control, INDO, OMEP and ICA (25)+INDO (50), respectively

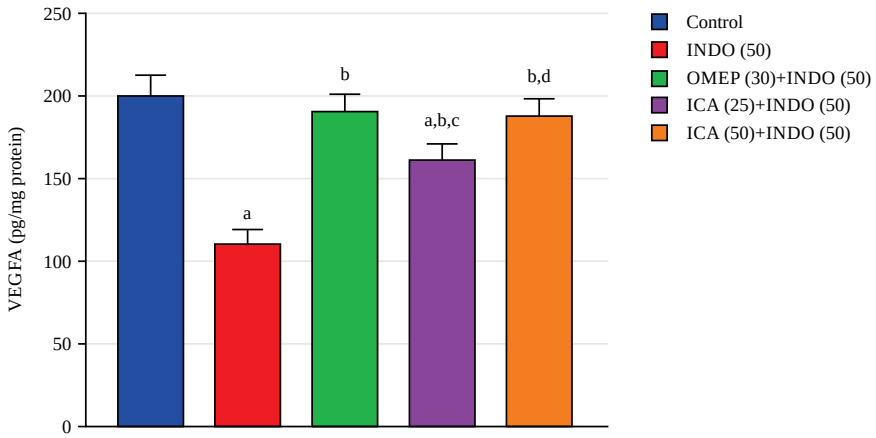

Fig. 5: Effect of ICA on angiogenic marker VEGFA

The values are displayed as Mean±SD (n = 6), [a,b,c,d]Statistically significant ($p<0.05$) differences compared to the control group, INDO (50), OMEP (30)+INDO (50) and ICA (25)+INDO (50), respectively

Effect of ICA on inflammatory markers: As shown in Fig. 4(a-d), the ingestion of INDO led to a substantial enhancement in the levels of IL-6, TNF-α, IL-1β and total NF-κβ p65 (266.9, 294, 315 and 457%, respectively) compared to the control group. Prior ingestion of ICA (25) led to a noteworthy stem in the levels of IL-6, TNF-α, IL-1β and total NF-κβ p65 by 42.40, 18.30, 25.62 and 38.85%, respectively, related to the INDO group. Pretreatment of ICA (50) resulted in reductions of their levels by 63.54, 50.25, 53.43 and 56.62%, respectively. Furthermore, the OMEP demonstrated a

substantial drop in the concentrations of IL-6, TNF-α, IL-1β and total NF-κβ p65 by 71, 67.32, 66 and 70%, correspondingly, relative to the INDO group.

Effect of ICA on angiogenic marker: The results shown in Fig. 5 indicate that the stomach tissue's VEGFA levels dropped significantly (45%) when exposed to INDO in comparison to the control. Pre-treatment with ICA (25 and 50) significantly raised VEGFA levels (47 and 71.30%, respectively), corresponding against the INDO group. Additionally, pretreatment of OMEP demonstrated an enormous spike in VEGFA content (73.15%) as compared to the INDO.

DISCUSSION

The present investigation unveiled that INDO caused substantial damage to the stomach mucosa, as indicated by macroscopic analysis. The aberration exhibited blood streaking and was associated with an elevation in the ulcer score. Pretreatment with ICA significantly diminished the stomach ulcer caused by INDO. A decrease in the ulcer index demonstrated this. Gastric ulcers are a common gastrointestinal circumstance worldwide[31]. A significant limitation of NSAIDs in clinical settings is the incidence of stomach ulcers[32]. The INDO has a higher likelihood of causing ulcers relative to other NSAIDs. Therefore, in an experimental setting, it is considered the preferred drug for causing damage to the stomach mucosa[33].

In addition, histopathological investigations provided evidence of the protective benefits of ICA. This finding was consistent with the previously reported ability of ICA to reduce the index of damage to the colon mucosa in rats[34].

Oxidative stress dramatically contributes to the formation of gastric ulcers[35]. Suleyman *et al*.[8] and Eraslan *et al*.[36] have established a connection between stomach ulcers produced by INDO and an oxidative imbalance. The present investigation observed a noteworthy reduction in SOD and CAT activities in the stomach tissues of rats administered INDO. However, there is a discernible increase in MDA levels. The MDA content was decreased while SOD and CAT activities were elevated following pretreatment with OMEP and ICA (25 and 50). These findings aligned with previous studies that demonstrate the ability of ICA to enhance antioxidant defence mechanisms, reduce lipid peroxidation and remove free radicals generated during oxidative stress[28,37,38]. Numerous studies have demonstrated that NSAIDs, such as INDO, have the potential to induce lipid peroxidation and inflict injury by generating reactive oxygen species (ROS)[39,40]. Lipid peroxidation byproducts like MDA are frequently employed to quantify oxidative strain in tissue and cell impairment[41]. The gut consists of enzymatic and nonenzymatic protective barriers which may diminish or avoid the injury caused to stomach tissue by an elevated level of ROS[42-44].

It was observed that the levels of PGE2 and COX-1 were considerably lowered after the administration of INDO. In contrast, the administration of ICA led to increased levels of COX-1 and PGE2. It is suggested that ICA treatment prevents stomach ulcers by restoring antioxidant processes and promoting the production of COX-1 and PGE2. The COX-1 is an important enzyme involved in the formation of PGE2[45]. The INDO inhibits the activity of COX-1, which leads to decreased levels of circulating PGE2[46]. According to Musumba *et al*.,[47] PGE2 has a major impact on mucus production and boosts gastric blood flow, which supports the stomach's defence system. The current study demonstrates that ingesting INDO resulted in elevated levels of IL-6, IL-1β, TNF-α and NF-κB in the gastric tissues. The result was consistent with prior studies conducted by Cho *et al*.[48] and Fu *et al*.[49]. Administration of INDO frequently results in inflammation in stomach tissues due to oxidative stress. The oxidative stress induces an elevation in reactive oxygen species (ROS), which triggers the synthesis of NF-κB[7]. Activation of NF-κB triggers the synthesis of several genes associated with inflammation, releasing TNF-α, IL-6, IL-12 and IL-1β. These cytokines have a crucial role in causing injury to epithelial cells and leading to the formation of stomach ulcers[7,50]. The ICA therapy effectively reduces inflammation by suppressing the activity of proinflammatory cytokines and NF-κB. This result aligns with the documented anti-inflammatory properties of ICA[28,37,38,51].

Angiogenesis is a crucial process in the repair of stomach ulcers. Vascular Endothelial Growth Factor (VEGF) is recognized as a highly effective promoter of angiogenesis[52]. The VEGF is essential for stimulating the development of granulation tissue and the creation of new tiny blood vessels[18,45]. The reduction in gastric VEGF levels was observed in rats treated with INDO which signifies delayed healing of the stomach ulcer produced by INDO. These outcomes perfectly align with previous studies[18,19,53]. Ingestion of ICA increased the level of VEGF suggesting that ICA possesses angiogenic properties and facilitates the healing process of the stomach ulcer. The current study findings were consistent with the ICA's known angiogenic qualities[54-56].

The findings provide valuable insights into the potential therapeutic applications of ICA. The study highlights ICA's

mechanisms, including its antioxidant, anti-inflammatory and pro-angiogenic properties, which collectively contribute to its protective effects against gastric ulcers. The study supports the exploration of ICA as a basis for developing new anti-ulcer medications or supplements, potentially offering a natural alternative to conventional treatments. If validated in clinical trials, ICA could be integrated into therapeutic regimens for patients with gastric ulcers or those at high risk of ulceration due to NSAID use.

Additional studies are necessary to confirm the safety and efficacy of ICA. Research should also investigate the optimal dosage and administration methods. Further exploration into the specific molecular pathways affected by ICA could enhance understanding of its therapeutic mechanisms and optimize its use in clinical settings. The study highlights the antioxidant, anti-inflammatory and pro-angiogenic properties of ICA, but it does not fully explore the detailed molecular mechanisms underlying these effects. Further research is needed to delineate these mechanisms precisely.

CONCLUSION

The research highlights the substantial gastroprotective benefits of ICA in averting INDO-induced stomach ulcers. Owing to its strong anti-inflammatory, antioxidant and angiogenic properties, ICA shows considerable therapeutic promise for the management of drug-induced gastrointestinal ulcers. The discoveries of the present research demonstrate the potential clinical value of ICA to mitigate stomach ulcers in addition to providing insight into the complex mechanisms that underlie its protective benefits. Therefore, more investigation and clinical testing are required to fully utilize ICA's therapeutic potential in treating gastric ulcer disease.

SIGNIFICANCE STATEMENT

The ICA, a flavonoid derived from Herba Epimedii, demonstrates potential therapeutic benefits in mitigating gastric ulcers induced by INDO in rats. This study highlights its protective effects against ulcer formation, suggesting its possible use as a novel treatment for preventing or managing (NSAIDs)-induced gastric damage. These findings open a new scope for optimizing ICA as a lead compound, further developing novel formulations of ICA to maximize its therapeutic potential activities.

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REFERENCES

1. Lanas, A. and F.K.L. Chan, 2017. Peptic ulcer disease. Lancet, 390: 613-624.
2. Ahmed, M.F., H. Thayyil, A.S. Rasheed and M. Ibrahim, 2010. Anti-ulcer activity of *Cassia auriculata* leaf extract. Pharmacogn. J., 2: 53-57.
3. Guzmán-Gómez, O., R.V. García-Rodríguez, S. Pérez-Gutierrez, N.L. Rivero-Ramírez and Y. García-Martínez *et al*., 2023. Protective effect of the phycobiliproteins from *Arthrospira maxima* on indomethacin-induced gastric ulcer in a rat model. Plants, Vol. 12. 10.3390/plants12081586.
4. Tamaddonfard, E ., A. Erfanparast, A.A. Farshid, M. Imani, N. Mirzakhani, R. Salighedar and S. Tamaddonfard, 2019. Safranal, a constituent of saffron, exerts gastro-protective effects against indomethacin-induced gastric ulcer. Life Sci., 224: 88-94.
5. Phillips, W.J. and B.L. Currier, 2004. Analgesic pharmacology: II. Specific analgesics. J. Am. Acad. Orthop. Surgeons, 12: 221-233.
6. Drina, M., 2017. Peptic ulcer disease and non-steroidal anti-inflammatory drugs. Aust. Prescriber, 40: 91-93.
7. Mahmoud, M.F., W. Abdo, M. Nabil, B. Drissi, A.M. El-Shazly, M.A.O. Abdelfattah and M. Sobeh, 2023. Apple (*Malus domestica* Borkh) leaves attenuate indomethacin-induced gastric ulcer in rats. Biomed. Pharmacother., Vol. 160. 10.1016/j.biopha.2023.114331.
8. Suleyman, H., A. Albayrak, M. Bilici, E. Cadirci and Z. Halici, 2010. Different mechanisms in formation and prevention of indomethacin-induced gastric ulcers. Inflammation, 33: 224-234.
9. Wang, T., S. Zhao, Y. Wang, Y. Yang and L. Yao *et al*., 2014. Protective effects of escin against indomethacin-induced gastric ulcer in mice. Toxicol. Mech. Methods, 24: 560-566.
10. Ma, N., Y. Sun, J. Yi, L. Zhou and S. Cai, 2022. Chinese sumac (*Rhus chinensis* Mill.) fruits alleviate indomethacin-induced gastric ulcer in mice by improving oxidative stress, inflammation and apoptosis. J. Ethnopharmacol., Vol. 284. 10.1016/j.jep.2021.114752.

11. Morsy, M.A. and M.A. El-Moselhy, 2013. Mechanisms of the protective effects of curcumin against indomethacin-induced gastric ulcer in rats. Pharmacology, 91: 267-274.
12. Zhou, D., Q. Yang, T. Tian, Y. Chang and Y. Li *et al*., 2020. Gastroprotective effect of gallic acid against ethanol-induced gastric ulcer in rats: Involvement of the Nrf2/HO-1 signaling and anti-apoptosis role. Biomed. Pharmacother., Vol. 126. 10.1016/j.biopha.2020.110075.
13. Benvenutti, R.C., C.A.D. Vecchia, G. Locateli, P.Z. Serpa and J.A. Lutinski *et al*., 2020. Gastroprotective activity of hydroalcoholic extract of the leaves of *Urera baccifera* in rodents. J. Ethnopharmacol., Vol. 250. 10.1016/j.jep.2019.112473.
14. Kim, Y.S., J.H. Lee, J. Song and H. Kim, 2020. Gastroprotective effects of inulae flos on HCl/ethanol-induced gastric ulcers in rats. Molecules, Vol. 25. 10.3390/molecules25235623.
15. Ahmed, M.A.E., M. Mohanad, A.A.E. Ahmed, B.E. Aboulhoda and S.A. El-Awdan, 2021. Mechanistic insights into the protective effects of chlorogenic acid against indomethacin-induced gastric ulcer in rats: Modulation of the cross talk between autophagy and apoptosis signaling. Life Sci., Vol. 275. 10.1016/j.lfs.2021.119370.
16. Athaydes, B.R., G.M. Alves, A.L.E.M. de Assis, J.V.D. Gomes and R.P. Rodrigues *et al*., 2019. Avocado seeds (*Persea americana* Mill.) prevents indomethacin-induced gastric ulcer in mice. Food Res. Int., 119: 751-760.
17. Liu, R., N. Zhu, Y. Hao, X. Liu and J. Kang *et al*., 2023. The protective effect of walnut oligopeptides against indomethacin-induced gastric ulcer in rats. Nutrients, Vol. 15. 10.3390/nu15071675.
18. Tijani, A.S., E.O. Farombi and S.B. Olaleye, 2021. Mechanisms underlying the healing potentials of the methanol extract of *Chasmanthera dependens* stem on indomethacin-induced gastric ulcer. Egypt. J. Basic Appl. Sci., 8: 17-31.
19. Shaik, R.A. and B.G. Eid, 2022. Piceatannol affects gastric ulcers induced by indomethacin: Association of antioxidant, anti-inflammatory, and angiogenesis mechanisms in rats. Life, Vol. 12. 10.3390/life12030356.
20. Jiang, J., J. Song and X.B. Jia, 2015. Phytochemistry and ethnopharmacology of *Epimedium* L. species. Chin. Herb. Med., 7: 204-222.
21. Zeng, Y., Y. Xiong, T. Yang, Y. Wang and J. Zeng *et al*., 2022. Icariin and its metabolites as potential protective phytochemicals against cardiovascular disease: From effects to molecular mechanisms. Biomed. Pharmacother., Vol. 147. 10.1016/j.biopha.2022.112642.
22. Jin, J., H. Wang, X. Hua, D. Chen, C. Huang and Z. Chen, 2019. An outline for the pharmacological effect of icariin in the nervous system. Eur. J. Pharmacol., 842: 20-32.
23. He, C., Z. Wang and J. Shi, 2020. Pharmacological Effects of Icariin. In: Advances in Pharmacology, Du, G. (Ed.), Academic Press, Cambridge, Massachusetts, ISBN: 9780128201855, pp: 179-203.
24. Tan, H.L., K.G. Chan, P. Pusparajah, S. Saokaew, A. Duangjai, L.H. Lee and B.H. Goh, 2016. Anti-cancer properties of the naturally occurring aphrodisiacs: Icariin and its derivatives. Front. Pharmacol., Vol. 7. 10.3389/fphar.2016.00191.
25. Shen, R. and J.H. Wang, 2018. The effect of icariin on immunity and its potential application. Am. J. Clin. Exp. Immunol., 7: 50-56.
26. Boivin, J., L. Bunting, J.A. Collins and K.G. Nygren, 2007. International estimates of infertility prevalence and treatment-seeking: Potential need and demand for infertility medical care. Hum. Reprod., 22: 1506-1512.
27. Sharma, S., V. Khan, N. Dhyani, A.K. Najmi and S.E. Haque, 2020. Icariin attenuates isoproterenol-induced cardiac toxicity in Wistar rats via modulating cGMP level and NF-κβ signaling cascade. Hum. Exp. Toxicol., 39: 117-126.
28. Aljehani, A.A., N.A. Albadr, M.Z. Nasrullah, T. Neamatallah, B.G. Eid and A.B. Abdel-Naim, 2022. Icariin ameliorates metabolic syndrome-induced benign prostatic hyperplasia in rats. Environ. Sci. Pollut. Res., 29: 20370-20378.
29. Sathish, R., B. Vyawahare and K. Natarajan, 2011. Antiulcerogenic activity of *Lantana camara* leaves on gastric and duodenal ulcers in experimental rats. J. Ethnopharmacol., 134: 195-197.
30. Neamatallah, T., 2024. Caffeic acid phenethyl ester attenuates indomethacin-induced gastric ulcer in rats. Naunyn-Schmiedeberg's Arch. Pharmacol., 397: 1791-1801.
31. Alfadil, A., 2024. Gastroprotective effect of 2,3-dimethylquinoxaline against indomethacin-induced gastric ulcer in rat. J. Inflammation Res., 17: 1983-1994.
32. Wu, J.Z., Y.H. Liu, J.L. Liang, Q.H. Huang and Y.X. Dou *et al*., 2018. Protective role of β-patchoulene from *Pogostemon cablin* against indomethacin-induced gastric ulcer in rats: Involvement of anti-inflammation and angiogenesis. Phytomedicine, 39: 111-118.
33. Ko, I.G., J.J. Jin, L. Hwang, S.H. Kim and C.J. Kim *et al*., 2020. Evaluating the mucoprotective effect of polydeoxyribonucleotide against indomethacin-induced gastropathy via the MAPK/NF-κβ signaling pathway in rats. Eur. J. Pharmacol., Vol. 874. 10.1016/j.ejphar.2020.172952.
34. Wang, Q.S., G.F. Wang, J. Zhou, L.N. Gao and Y.L. Cui, 2016. Colon targeted oral drug delivery system based on alginate-chitosan microspheres loaded with icariin in the treatment of ulcerative colitis. Int. J. Pharm., 515: 176-185.
35. Bhattacharyya, A., R. Chattopadhyay, S. Mitra and S.E. Crowe, 2014. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev., 94: 329-354.

36. Eraslan, E., A. Tanyeli, M.C. Güler, N. Kurt and Z. Yetim, 2020. Agomelatine prevents indomethacin-induced gastric ulcer in rats. Pharmacol. Rep., 72: 984-991.
37. El-Shitany, N.A. and B.G. Eid, 2019. Icariin modulates carrageenan-induced acute inflammation through HO-1/Nrf2 and NF-κβ signaling pathways. Biomed. Pharmacother., Vol. 120. 10.1016/j.biopha.2019.109567.
38. Algandaby, M.M., R.M. Breikaa, B.G. Eid, T.A. Neamatallah, A.B. Abdel-Naim and O.M. Ashour, 2017. Icariin protects against thioacetamide-induced liver fibrosis in rats: Implication of anti-angiogenic and anti-autophagic properties. Pharmacol. Rep., 69: 616-624.
39. Harakeh, S., S.H. Saber, I.O. Akefe, S. Shaker and M.B. Hussain *et al*., 2022. Saudi honey alleviates indomethacin-induced gastric ulcer via improving antioxidant and anti-inflammatory responses in male albino rats. Saudi J. Biol. Sci., 29: 3040-3050.
40. Jafari, A., N. Andishfar, Z. Esmaeilzadeh, M.R. Khezri and M. Ghasemnejad-Berenji, 2022. Gastroprotective effect of topiramate on indomethacin-induced peptic ulcer in rats: Biochemical and histological analyses. Basic Clin. Pharmacol. Toxicol., 130: 559-568.
41. Tsikas, D., 2017. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal. Biochem., 524: 13-30.
42. Ruiz-Hurtado, P.A., L. Garduño-Siciliano, P. Dominguez-Verano, E. Martinez-Galero, M.M. Canales-Martinez and M.A. Rodriguez-Monroy, 2021. Evaluation of the gastroprotective effects of *Chihuahua propolis* on indomethacin-induced gastric ulcers in mouse. Biomed. Pharmacother., Vol. 137. 10.1016/j.biopha.2021.111345.
43. Chen, X.Y., H.M. Chen, Y.H. Liu, Z.B. Zhang and Y.F. Zheng *et al*., 2016. The gastroprotective effect of pogostone from pogostemonis herba against indomethacin-induced gastric ulcer in rats. Exp. Biol. Med., 241: 193-204.
44. Toktay, E., M. Yayla, L. Sahin, A. Harmankaya and C. Ozic *et al*., 2022. The effects of dragon fruit (*Hylocereus polyrhizus*) extract on indomethacin-induced stomach ulcer in rats. J. Food Biochem., Vol. 46. 10.1111/jfbc.14274.
45. Adhikary, B., S.K. Yadav, K. Roy, S.K. Bandyopadhyay and S. Chattopadhyay, 2011. Black tea and theaflavins assist healing of indomethacin-induced gastric ulceration in mice by antioxidative action. Evidence-Based Complementary Altern. Med., Vol. 2011. 10.1155/2011/546560.
46. Takeuchi, K., 2012. Pathogenesis of NSAID-induced gastric damage: Importance of cyclooxygenase inhibition and gastric hypermotility. World. J. Gastroenterol., 18: 2147-2160.
47. Musumba, C., D.M. Pritchard and M. Pirmohamed, 2009. Review article: Cellular and molecular mechanisms of NSAID-induced peptic ulcers. Aliment. Pharmacol. Ther., 30: 517-531.
48. Cho, H.S., T.W. Kwon, J.H. Kim, R. Lee and C.S. Bae *et al*., 2023. Gintonin alleviates HCl/ethanol- and indomethacin-induced gastric ulcers in mice. Int. J. Mol. Sci., Vol. 24. 10.3390/ijms242316721.
49. Fu, Y., H.Q. Wu, H.L. Cui, Y.Y. Li and C.Z. Li, 2018. Gastroprotective and anti-ulcer effects of oxymatrine against several gastric ulcer models in rats: Possible roles of antioxidant, antiinflammatory, and prosurvival mechanisms. Phytother. Res., 32: 2047-2058.
50. Ermis, A., G.A. Colak, M. Acikel-Elmas, S. Arbak and M. Kolgazi, 2023. Ferulic acid treats gastric ulcer via suppressing oxidative stress and inflammation. Life, Vol. 13. 10.3390/life13020388.
51. Chen, S.R., X.Z. Xu, Y.H. Wang, J.W. Chen, S.W. Xu, L.Q. Gu and P.Q. Liu, 2010. Icariin derivative inhibits inflammation through suppression of p38 mitogen-activated protein kinase and nuclear factor-κB pathways. Biol. Pharm. Bull., 33: 1307-1313.
52. Takahashi, M., S. Maeda, K. Ogura, A. Terano and M. Omata, 1998. The possible role of vascular endothelial growth factor (VEGF) in gastric ulcer healing: Effect of sofalcone on VEGF release *in vitro*. J. Clin. Gastroenterol., 27: S178-S182.
53. Nur Azlina, M.F., H.M.S. Qodriyah, K.H. Chua and Y. Kamisah, 2017. Comparison between tocotrienol and omeprazole on gastric growth factors in stress-exposed rats. World J. Gastroenterol., 23: 5887-5894.
54. Chung, B.H., J.D. Kim, C.K. Kim, J.H. Kim and M.H. Won *et al*., 2008. Icariin stimulates angiogenesis by activating the MEK/ERK- and PI3K/Akt/eNOS-dependent signal pathways in human endothelial cells. Biochem. Biophys. Res. Commun., 376: 404-408.
55. Li, X., Y. Wen, L. Sheng, R. Guo, Y. Zhang and L. Shao, 2022. Icariin activates autophagy to trigger TGFβ1 upregulation and promote angiogenesis in EA.hy926 human vascular endothelial cells. Bioengineered, 13: 164-177.
56. Wu, Y., L. Xia, Y. Zhou, W. Ma and N. Zhang *et al*., 2015. Evaluation of osteogenesis and angiogenesis of icariin loaded on micro/nano hybrid structured hydroxyapatite granules as a local drug delivery system for femoral defect repair. J. Mater. Chem. B, 3: 4871-4883.