ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 2, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

ANTI-ULCEROGENIC EFFECT OF GENISTEIN AGAINST INDOMETHACIN-INDUCED GASTRIC ULCER IN RATS

IHAB T ABDEL-RAHEEM[1,2]*, GHAZI A BAMAGOUS[3], GAMAL A OMRAN[4]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Damanhour University, Damanhour 22111, Egypt. [2]Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia. [3]Department of Pharmacology and Toxicology, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia. [4]Department of Biochemistry, Faculty of Pharmacy, Damanhour University, Damanhour 22111, Egypt. Email: Ihabpharma@yahoo.com

Received: 16 September 2015, Revised and Accepted: 21 January 2016

ABSTRACT

Objectives: Free radicals generation, inflammation, and nitric oxide (NO) modulation are involved in indomethacin (IND)-induced gastric ulcers. Most of the drugs used for the treatment of gastric ulcer have various side effects. Genistein (GEN), the natural isoflavones isolated from soya bean has an antioxidant, anti-inflammatory, and NO modulating activity. These properties could make GEN a promising and safe, natural candidate against IND-induced peptic ulcers.

Methods: Rats were divided into four groups. Control group; GEN group (10 mg/kg, p.o.); IND group (48 mg/kg, orally); and GEN+IND group (GEN administered 1 week before IND injection). 6 hrs after IND administration, all rats were sacrificed. Gastric juice acidity and gastric injury were evaluated directly. The levels of tumor necrosis factor alpha (TNF-α), myeloperoxidase (MPO), and NO were determined in gastric tissues. Moreover, glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD) activity were determined in mucosal tissues.

Results: Several mechanisms are involved in IND-induced ulcers as evidenced from the increase in TNF-α, MPO, and reduction in NO levels. In addition, free radicals also participate in the pathogenesis as evidenced from the increase in MDA levels and reduction in GSH levels and SOD activity. On the other hand, pre-administration of GEN markedly attenuated IND-induced ulcers without affecting gastric acidity, through a reduction of the elevated TNF-α and MPO levels. Moreover, GEN significantly restored the declined NO level and ameliorated the unbalanced oxidative stress parameters.

Conclusion: GEN markedly protects against IND-induced ulcers as revealed from biochemical data and amelioration of IND-induced lesions. Therefore, GEN may be a promising candidate for protection against IND-induced gastropathy.

Keywords: Genistein, Ulcers, Indomethacin, Nitric oxide, Myeloperoxidase, Tumor necrosis factor alpha.

INTRODUCTION

Gastric ulcer is a painful lesion that is caused by erosion of the mucosal layer of stomach or necrosis of the surface of a tissue due to sloughing of inflammatory necrotic tissue [1]. In normal situations, stomach maintains a balance between protective factors and aggressive factors [2]. When the balance between aggressive factors (such as acid and pepsin) and defense mechanisms (such as mucus, bicarbonate, blood flow, and mucosal turnover) is shifted in favor of aggressive factors, peptic ulcers are manifested [3]. Factors, such as stress, smoking, alcohol usage, nutritional deficiencies, and frequent ingestion of non-steroidal-anti-inflammatory drugs (NSAIDs), have been shown to be associated with an increased incidence of gastric ulcer [4]. Several mechanisms to explain the pathophysiology of gastric ulceration development associated with frequent NSAID use have been suggested, including inhibition of prostaglandin synthesis [5] and increased mucosal production of inflammatory cytokines (e.g. tumor necrosis factor alpha [TNF-α]) [6]. TNF-α is a well-characterized stimulus for adhesion molecule expression [7]. Appleyard *et al*. [8] suggested that TNF-α might be the key signal for NSAID-induced neutrophil adherence within the gastric microcirculation. Other possible mechanisms of NSAIDs-induced gastropathy include overproduction of reactive oxygen species and lipid peroxidation [9]. In addition, it was reported that the increased ulcerogenic response to indomethacin (IND) is mediated through the reduction of constitutive nitric oxide synthase (cNOS) activity [10]. Overall, it is obvious that many factors are involved in the pathogenesis of IND-induced gastric ulcers such as leukocyte infiltration, free radicals formation, and disturbance in NO production in gastric tissues. Therefore, any compound isolated from natural sources and exhibits antioxidant properties and has the capacity to inhibit neutrophil infiltration as well as modify NO level might be of good value in the protection against IND-induced gastric ulcer.

Genistein (GEN), a soy-derived isoflavone, has received a huge attention as it has been demonstrated to be effective in the prevention of today's most prevalent chronic diseases, namely cardiovascular diseases, osteoporosis, and hormone-related cancers [11]. GEN is widely available in the Asian diet and is believed to be a potent antioxidant [12]. Scientists believe that isoflavones, by their antioxidant nature, are capable of neutralizing free radicals and reducing the inflammatory reactions [12]. In addition, GEN has been demonstrated to activate peroxisome proliferator-activated receptors PPARs [13], a subgroup of the nuclear hormone receptor superfamily of ligand-activated transcription factors that are critical in regulating insulin sensitivity, adipogenesis, lipid metabolism, inflammation, and blood pressure. Furthermore, in double-blinded, randomized, clinical trials, GEN was proven to increase plasma NO breakdown products, decrease endothelin-1 levels, and enhance endothelial-dependent vasodilatation [14]. It also reduced homocysteine and C-reactive protein levels [15]. GEN by its multi-mechanisms is documented to be effective in the prevention of cardiovascular and renal diseases; however, its role in NSAI-induced peptic ulcer is not tested yet.

The aim of this study was to investigate the anti-ulcerative effect of GEN against IND-induced gastric ulcer and whether it is linked to oxidant/antioxidant/anti-inflammatory effects. Accordingly, ulcer index, myeloperoxidase (MPO), and NO were measured in gastric tissues. In addition, oxidative stress biomarkers such as reduced glutathione (GSH), lipid peroxidation (malondialdehyde [MDA]) levels, superoxide

dismutase (SOD) activity, and histopathological changes were evaluated in gastric mucosal tissues of rats treated with IND alone or combination of IND and GEN.

MATERIALS AND METHODS

Animals

Adult male Wistar albino rats weighing 140-160 g were selected for this study. Rats were obtained from the national research center, Egypt. They were fed standard diet and water *ad-libitum*. During the study, rats were maintained at 12 hrs light/dark cycle. They were kept in a fasting state for 24 hrs before the experiments but had free access to drinking water. All the experiments were performed in accordance with international institutional guidelines for the ethical care of animals. The study protocol was approved by research ethics committee, Faculty of Pharmacy, Damanhour University, Egypt.

Drugs and chemicals

GEN was purchased from Sigma (St. Louis, MO, USA). IND was obtained from Nile Co., for Pharmaceutical Industries (Cairo, Egypt). All other chemicals used were of good quality and analytical grade.

Experimental protocol

The animals were divided into 4 groups, each of 8 rats:

Group 1: Rats in this group (control) were given the vehicles orally by intragastric gavage.

Group 2: Rats in this group were intragastrically treated with GEN (10mg/kg) [16].

Group 3: Rats in this group were treated orally by an intragastric tube with 48 mg/kg of IND dissolved in 5% $NaHCO_3$. This dose of IND is within the range reported in the literature to elicit macroscopically visible acute hemorrhagic erosions and ulcers in the stomach of normal rats [17].

Group 4: Rats in this group were 1 week pretreated with GEN orally [16] before IND at the same previous doses.

After 6 hrs of IND treatment, the rats were sacrificed by decapitation; their stomachs were rapidly removed and processed for evaluation of gastric mucosal damage and histopathological examination. The gastric mucosa was rapidly scraped using two glass slides from the gastric wall of each rat and immediately weighed and homogenized in ice-cold 0.1 M Tris-HCl buffer (pH 7.5). The aliquot was kept frozen until the determination of biochemical parameters such as TNF-α, MPO, total nitrite/nitrate levels, and oxidative stress biomarkers (GSH, MDA contents, and SOD activity) in gastric mucosal tissues.

Animals subjected to pylorus ligation

To examine the effects of IND and or GEN on gastric secretion, studies were performed in different groups of pylorus ligated rats as described by Rajashekhara *et al*. [18]. Four groups of animals (n=6) were used in this experiment. Under short anesthesia with diethyl ether, the abdomen was opened by a midline laparotomy and the duodenum was exteriorized. The pylorus was ligated, the abdominal incision was closed with clips, and the animals were allowed to recover from anesthesia. The first control group was given the vehicle orally. The second group was treated orally with GEN (10 mg/kg). The third group was given IND 48 mg/kg orally; the fourth group was pretreated orally with GEN for 1 week before IND. The animals were sacrificed at 6 hrs following IND administration. The contents of stomachs were emptied; the collected volume was measured, centrifuged, and analyzed for acidity using 0.01N NaOH. Units are expressed as mEq/l.

Evaluation of gastric mucosal injury

The stomachs were collected, opened, and washed with saline, pinned out on a wax platform, and photographed and the gastric lesions were measured blindly to determine the ulcer index. The gastric ulcer index was then calculated as the sum of the lengths in millimeters of all lesions (seen as red streaks) [19].

Determination of gastric mucosal TNF-α concentration

Gastric tissue TNF-α concentration was measured in mucosal homogenate using an enzyme-linked immunosorbent assay kit (rat TNF-α ELISA kit, Biosource, Belgium) according to the manufacturer's protocol. TNF-α concentrations expressed as pg/g tissue.

Determination of gastric mucosal MPO concentration

MPO concentration was determined in rat gastric tissue homogenate using rat enzyme-linked immunosorbent assay kit (rat MPO ELISA kit, Wkea Med Supplies Corp, China) according to manufacturer protocol. MPO concentrations expressed as ng/g tissue.

Determination of total nitrite/nitrate contents in gastric mucosal tissues

NO was estimated in gastric mucosal tissues by commercially available NO assay kit (Biodiagnostic, Dokki, Giza, Egypt). The principle of its estimation is based on, in acidic medium and in the presence of nitrite, the formed nitrous acid diazotizes sulfanilamide, and the product is coupled with N-(1–naphthyl) ethylenediamine. The bright, reddish-purple color of the azo dye can be measured at 540 nm.

Estimation of oxidative stress biomarkers in gastric mucosal tissues

GSH content of gastric tissues was determined in the homogenates using commercially available GSH assay kit. Lipid peroxidation was calculated by estimating the level of MDA. MDA was determined in rat gastric homogenates using commercially available MDA assay kit. The results were expressed as nmol MDA/g tissue. The enzymatic activity of SOD was assessed in gastric tissue homogenate using commercially available SOD assay kit. SOD activity was determined by calculating the difference between auto-oxidation of pyrogallol alone and in the presence of SOD enzyme. GSH, MDA, and SOD assay kits were obtained from Biodiagnostic, Dokki, Giza, Egypt.

Histopathological evaluation

The stomach of each animal was removed, fixed in 10% formalin for 24 hrs and processed for histopathological examination. Four-micrometer-thick paraffin sections were stained with hematoxylin and eosin for light microscope examination using conventional protocol [20]. A minimum of 8 fields for each stomach section were examined and assigned for the severity of changes by an observer blinded to the treatments of the animals.

Statistical analysis

Data were expressed as the means±SE. One-way analysis of variance (ANOVA) test followed by Tukey's *post-hoc* test was used to determine the statistically significant difference between multiple comparisons. A probability value of $p<0.05$ was considered to indicate statistical significance.

RESULTS

Gastric ulcer index and gastric juice acidity

The gastric ulcer index is calculated as the sum of the lengths in mm of all hemorrhagic and ulcerative lesions. The ulcer index (mm) measured in the gastric mucosa of the IND-treated group was (45±2.45). Administration of GEN markedly reduced the ulcer index to (15±1.42) resulting in 66.67% reduction in gastric lesions induced by IND in rat stomachs ($p<0.001$) (Fig. 1a). Oral administration of GEN alone to rats did not show hemorrhagic or ulcerative lesions (Fig. 1a). In terms of gastric juice acidity, IND increased gastric mucosal acidity (mEq/l) to give a value of (101±9.3) compared to control value of (35±1.66). Pre-treatment of rats with GEN showed no significant effect in gastric acidity compared to IND group (Fig. 1b). Oral administration of GEN alone also did not affect gastric acidity (Fig. 1b).

Quantitative estimation of gastric mucosal contents of TNF-α, MPO, and NO expressed as total nitrite/nitrate.

Oral administration of IND significantly increased the pro-inflammatory mediator TNF-α by more than 3 folds as compared with the control group (Fig. 2a). Pre-administration of GEN before IND markedly reduced the elevated TNF-α contents by 50 % ($p<0.001$) (Fig. 2a). Rats treated with IND orally showed an increase in MPO contents to give a value of (40±1.3) compared to the control group (12±0.93) indicating enhancement of neutrophils infiltration into mucosal tissues. Administration of GEN one week before IND reduced the elevated MPO level to (23±0.94) ($p<0.001$) (Fig. 2b). In addition, NO level expressed as total nitrite/nitrate (nmol/g tissue) was lowered by about 46% to give a value of (350±22.2) compared to control (651±21) after IND administration. Pre-treatment with GEN significantly elevated the decreased total nitrite/nitrate levels in gastric mucosa to (577±22) ($p<0.001$) (Fig. 2c).

Determination of oxidative stress biomarkers (GSH, MDA, and SOD) in gastric mucosal tissues

IND in this study produced a decrease in GSH levels (nmol/g tissue) from 23±1.5 to reach 12±1. GEN prevented the IND-induced decline in reduced GSH content and restored its normal level to 20±1.6 ($p<0.05$) (Fig. 3a). Moreover, IND caused an elevation in lipid peroxide (MDA) levels (nmol/g tissue) in gastric tissues from 1.5±0.1 to 8±0.6. GEN was able to normalize the elevated MDA levels to 2±0.16 ($p<0.001$) (Fig. 3b). SOD activity (U/mg protein) was decreased in gastric tissues of IND-treated rats from 15±1.3 to 8.5±0.46. However, the reduced SOD activity was increased to 12±1.1 following GEN administration ($p<0.05$) (Fig. 3c). Administration of GEN (alone) did not show any significant effects on GSH, MDA contents or SOD activity, respectively (Fig. 3a-c).

Assessment of gastric mucosal injury morphologically and histologically

No visible hemorrhagic and/or ulcerative lesions were seen in the control and GEN groups (Fig. 4a and c). Sections from control and GEN groups showed the normal histological structure of the gastric mucosa glandular and non-glandular layers (Fig. 4b and d). Oral administration of IND for 6 hrs induced a gross gastric mucosal damage in rats. These hemorrhagic and ulcerative lesions were seen as red streaks (Fig. 4e). Histologically, this group showed erosion associated with diffuse leukocyte cells infiltration that was observed in lamina propria of the mucosal layer. This was accompanied by hypertrophy in the muscularis of the glandular portion (Fig. 4f). Pre-treatment with GEN markedly reduced the visible hemorrhagic lesions induced by IND in rats' stomach (Fig. 4g). Histologically, pre-treatment with GEN before IND maintained the normal histological structure of glandular portion (Fig. 4h) with mild edema in the junction between glandular and non-glandular portions (Fig. 4h). Most of the histopathological lesions induced by IND and ameliorated by GEN in gastric tissues were summarized in Table 1.

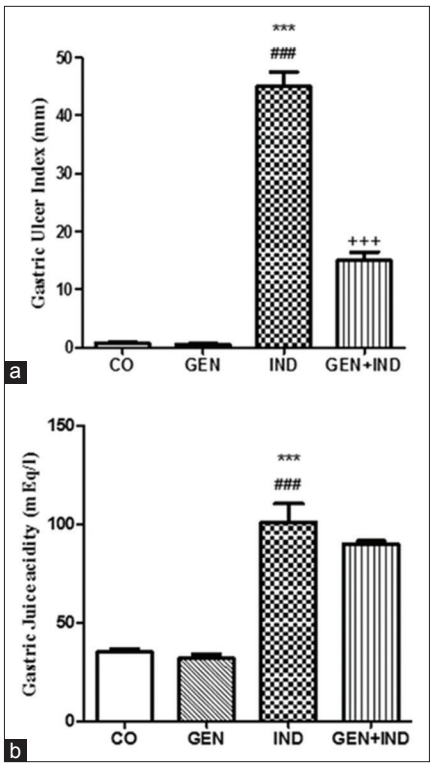

Fig. 1: Effects of genistein (GEN), indomethacin (IND) and their combination (GEN+IND) on gastric ulcer index (a), gastric juice acidity (b) levels in rats compared to control (CO) group. The significant difference between two groups was determined by ANOVA followed by Tukey's multiple comparison test. ***p<0.001: Statistically significant difference from CO group. ###p<0.001: Statistically significant difference from GEN group. +++p<0.001: Statistically significant difference from IND group

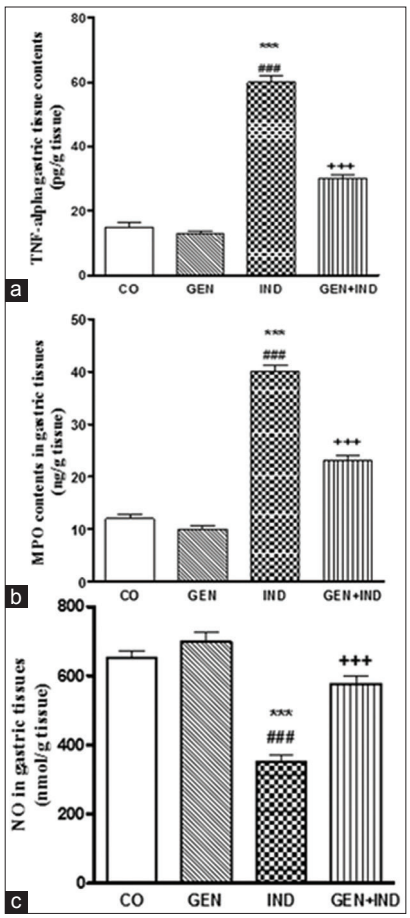

Fig. 2: Effects of genistein (GEN), indomethacin (IND) and their combination (GEN+IND) on tumor necrosis factor-alpha (a), myeloperoxidase (MPO) (b) and nitric oxide (c) levels in rats compared to control (CO) group. The significant difference between two groups was determined by ANOVA followed by Tukey's multiple comparison test. ***p<0.001: Statistically significant difference from CO group. ###p<0.001: Statistically significant difference from GEN group. +++p<0.001: Statistically significant difference from IND group

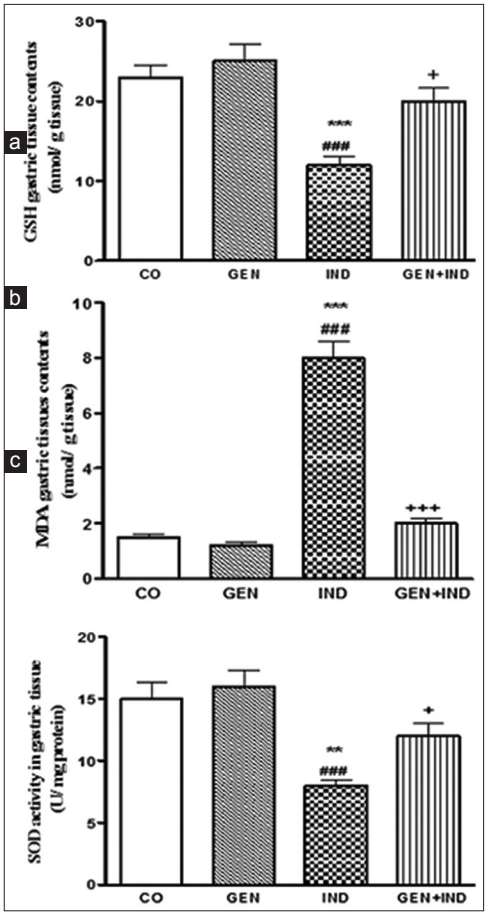

Fig. 3: Effects of genistein (GEN), indomethacin (IND) and their combination (GEN+IND) on reduced glutathione, (a) malondialdehyde (MDA), (b) levels and superoxide dismutase (SOD) (c) activity in rats compared to control (CO) group. The significant difference between two groups was determined by ANOVA followed by Tukey's multiple comparison test. **p<0.01: Statistically significant difference from CO group. ***p<0.001: Statistically significant difference from CO group. ###p<0.001: Statistically significant difference from GEN group. +p<0.05: Statistically significant difference from IND group. +++p<0.001: Statistically significant difference from IND group

Table 1: The severity of histopathological alterations in gastric tissues of experimental groups of rats treated with GEN, IND and their combination (GEN+IND) in comparison to the control group (CO)

Groups/histopathological alterations	CO	GEN	IND	GEN+IND
Congestion	0	0	+++	+
Hemorrhage	0	0	+++	0
Inflammatory cells infiltrations	0	0	+++	0
Muscular hypertrophy	0	0	++	0
Edema	0	0	+++	+

GEN: Genistein, IND: Indomethacin, +: Mild level, ++: Moderate level, +++: Severe level

Data were obtained from investigation of four histological sections from the gastric tissues of the control group (CO), GEN-treated group, IND-treated group, and their combination (GEN+IND). The severity of changes using scores of 0 (absent) and + (mild level): <25% of the total fields examined revealed histopathological alterations. ++ (moderate level): <50% of the total fields examined revealed histopathological alterations. +++ (severe level): <75% of the total fields examined revealed histopathological alterations.

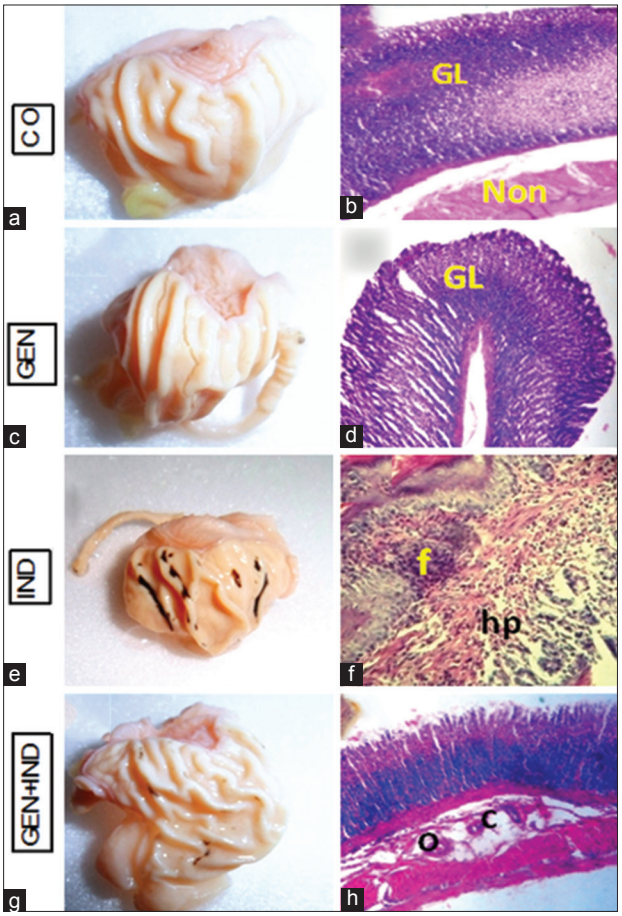

Fig. 4: The protective effects of genistein (GEN) morphologically and histologically against gastric mucosal hemorrhagic lesions induced by indomethacin (IND) in rats. No visible hemorrhagic and ulcerative lesions were seen in control (CO) and GEN groups (a and c). Sections from control and GEN groups showed the normal histological structure of the gastric mucosa glandular and non-glandular layers (b and d). Oral administration of IND for 6 hrs induced gross gastric mucosal damage in rats. These hemorrhagic and ulcerative lesions were seen as red streaks (e). Histologically, this group showed erosion associated with diffuse leukocyte cells infiltration (f) that was observed in lamina propria of the mucosal layer. This is accompanied by hypertrophy (hp) in the muscularis of the glandular portion (f). Pre-treatment with GEN markedly reduced the visible hemorrhagic lesions induced by IND in rat stomachs (g). Histologically, pre-treatment with GEN before IND maintained the normal histological structure of glandular portion (h) with mild edema (o) and congestion (c) in the junction between glandular and non-glandular portions (h)

DISCUSSION

NSAIDs are valuable agents in the treatment of arthritis and other musculoskeletal disorders, and as analgesics in a wide variety of clinical scenarios. Unfortunately, their use has been limited by their association with mucosal injury to the upper gastrointestinal tract, including the development of peptic ulcer disease along with its complications, most notably upper gastrointestinal hemorrhage, and perforation [21]. IND is an NSAIDs that was reported to induce gastric ulcer through multi-mechanisms [22].

Our results showed that IND produced gastric ulcer in rats as indicated from gastric ulcer index. According to Sabiu *et al*. [22], the abnormal elevation of gastric juice acidity by IND may also participate in the augmentation of the severity of gastric ulcer. Choi *et al*. [23] reported that inflammatory mediators, such as TNF-α, are involved in the pathogenesis of IND-induced gastric ulcer and is one of the aggressive factors in ulcerogenesis. It was documented that TNF-α level is elevated in gastric tissues of IND-treated rats [23], and this came online with our findings. Furthermore, it was proved that there is a relation between TNF-α and PGE2. It was found that exogenous administration of PGE2 produced an anti-ulcer effect by preventing the IND-induced TNF-α increase [24].

There is evidence that TNF-α stimulates neutrophil infiltration into the gastric mucosa, and its overproduction increases the risk of gastric ulcer and cancer [24]. MPO (a neutrophil infiltration index) level is augmented following IND administration suggesting a role of TNF-α in neutrophil cells infiltration and in the development of gastric ulcer [25]. On the other hand, NO produced by eNOS or cNOS plays a cytoprotective role against any injuries insults that harm the gastric tissues possibly through a reduction of leukocyte adhesion and maintenance of gastric blood flow and any reduction in its content will expose the tissues to the damaging effects of IND [25]. Our results are matched with the aforementioned findings as IND administration to rats has led to an increase in MPO levels and a reduction in NO levels.

Another mechanism that is involved in the pathogenesis of gastric ulcer is oxidative stress mechanism. Based on certain studies, IND causes an overproduction of ROS [25,26]. This was similar to the findings of this study in which the administration of IND to rats reduced the GSH levels and SOD activity in gastric tissues. This was also consistent with what was reported by Kaplan *et al*. [27]. On the other hand, IND elevated the MDA level in this study; MDA is accepted as a marker of lipid oxidation [25].

GEN, a class of phytoestrogens, known as isoflavones, is mostly found in legumes. It has attracted attention because of its beneficial effects on the prevention of metabolic disorders related to cardiovascular disease (CVD), obesity, cancer, and diabetes [28,29]. GEN has been extensively established as a multifunctional agent through enhancing the antioxidant defense system and anti-inflammation response [30]. Obviously, GEN in this study has showed a protective effect against IND-induced ulcers in rats. Pre-treatment with GEN significantly reduced the gastric ulcer index (mm) that was elevated after IND treatment. Gastric ulcer index is the measure of hemorrhagic and ulcerative lesions that are produced by IND in gastric tissues. Pro-inflammatory mediators such as TNF-α are involved in the pathogenesis of gastric ulcers. GEN was reported to decline the elevated TNF-α in d-galactosamine induced fulminant hepatic failure in Wistar rats [31], an effect that came in agreement with our study as the administration of GEN markedly reduced the elevated TNF-α levels in gastric tissues. The infiltration of neutrophils (measured as MPO index) into ischemic myocardium exacerbates myocardial damage on reperfusion [32], whereas drugs such as GEN that inhibit neutrophils activity or function [33] may reduce the inflammatory damaging effect that seen after neutrophils infiltration. In our study, pre-treatment with GEN reduced the MPO activity, giving an indication that GEN is a neutrophils activity inhibitor. Therefore, GEN may inhibit infiltration of neutrophils into gastric tissues, which produce and secrete TNF-α leading to a reduction in the pro-inflammatory mediators (TNF-α) release into gastric tissues. NO produced in gastric tissues has a cytoprotective role. GEN pre-treatment enhanced NO levels that were reduced by IND in gastric tissues. Wang *et al*. [34] reported that GEN augments NO levels in rats subjected to four-vessel global cerebral ischemia through enhancement of eNOS phosphorylation/activation, supporting our results and emphasizing the role of NO in gastric protection against IND gastric lesion.

Oxidative stress mechanism is also involved in the pathogenesis of gastric ulcer induced by IND. GEN is reported to have an antioxidant mechanism [35]. GEN was found to increase GSH levels and SOD activity and reduced the elevated MDA levels in mouse liver [36]. Our results are matched with the aforementioned findings, GEN increased GSH levels, SOD activity and reduced the elevated MDA contents in gastric tissues that were deteriorated after IND treatment.

Based on the above findings, GEN ameliorated the IND-induced gastric ulcers through multi-mechanisms as indicated from the improvement of biochemical analysis. Our results are further supported by the histopathological analysis that reveals alleviation of pathological lesions (e.g., erosion, diffuse leukocyte cells infiltration, and hypertrophy in the muscularis of the glandular portion) after treatment with GEN. In contrast to what is expected, GEN has no effect on the gastric acidity that was elevated by IND, indicating that GEN has a gastro-protective effect without effect on gastric acidity.

CONCLUSION

These results indicate that GEN has a protective effect against IND-induced gastropathy probably through inhibiting inflammatory mediators, neutrophil infiltration, suppression of oxidative stress generation and replenishing of NO levels regardless of gastric acidity.

ACKNOWLEDGMENTS

We are grateful to Prof. Adel M. Bakeer, a professor of pathology, Faculty of Veterinary Medicine, Cairo University for his kind help in performing histopathological studies and interpretation of the results.

REFERENCES

1. Yuan Y, Padol IT, Hunt RH. Peptic ulcer disease today. Nat Clin Pract Gastroenterol Hepatol 2006;3(2):80-9.
2. Araújo MB, Borini P, Guimarães RC. Etiopathogenesis of peptic ulcer: Back to the past? Arq Gastroenterol 2014;51(2):155-61.
3. Lima ZP, Severi JA, Pellizzon CH, Brito AR, Solis PN, Cáceres A, *et al*. Can the aqueous decoction of mango flowers be used as an antiulcer agent? J Ethnopharmacol 2006;106(1):29-37.
4. Belaiche J, Burette A, De Vos M, Louis E, Huybrechts M, Deltenre M; Belgian Study Group of NSAID-GI Complications. Observational survey of NSAID-related upper gastro-intestinal adverse events in Belgium. Acta Gastroenterol Belg 2002;65(2):65-73.
5. Dhiyaaldeen SM, Amin ZA, Darvish PH, Mustafa IF, Jamil MM, Rouhollahi E, *et al*. Protective effects of (1-(4-hydroxy-phenyl)-3-m-tolyl-propenone chalcone in indomethacin-induced gastric erosive damage in rats. BMC Vet Res 2014;10(6):961.
6. Abbas AM, Sakr HF. Effect of selenium and grape seed extract on indomethacin-induced gastric ulcers in rats. J Physiol Biochem 2013;69(3):527-37.
7. Mitra RS, Shimizu Y, Nickoloff BJ. Histamine and cis-urocanic acid augment tumor necrosis factor-alpha mediated induction of keratinocyte intercellular adhesion molecule-1 expression. J Cell Physiol 1993;156(2):348-57.
8. Appleyard CB, McCafferty DM, Tigley AW, Swain MG, Wallace JL. Tumor necrosis factor mediation of NSAID-induced gastric damage: Role of leukocyte adherence. Am J Physiol 1996;270:G42-8.
9. Handa O, Majima A, Onozawa Y, Horie H, Uehara Y, Fukui A, *et al*. The role of mitochondria-derived reactive oxygen species in the pathogenesis of non-steroidal anti-inflammatory drug-induced small intestinal injury. Free Radic Res 2014;48(9):1095-9.
10. Slomiany BL, Slomiany A. Role of endothelin-converting enzyme-1 in the suppression of constitutive nitric oxide synthase in rat gastric mucosal injury by indomethacin. Scand J Gastroenterol 2000;35(2):1131-6.
11. Vitale DC, Piazza C, Melilli B, Drago F, Salomone S. Isoflavones: Estrogenic activity, biological effect and bioavailability. Eur J Drug Metab Pharmacokinet 2013;38(30):15-25.
12. Saleh DO, Abdel Jaleel GA, El-Awdan SA, Oraby F, Badawi M. Thioacetamide-induced liver injury: Protective role of genistein. Can J Physiol Pharmacol 2014;92(11):965-73.
13. Xiang Q, Lin G, Fu X, Wang S, Wang T. The role of peroxisome proliferator-activated receptor-gamma and estrogen receptors in genistein-induced regulation of vascular tone in female rat aortas. Pharmacology 2010;86(2):117-24.
14. Squadrito F, Altavilla D, Morabito N, Crisafulli A, D'Anna R, Corrado F, *et al*. The effect of the phytoestrogen genistein on plasma

nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women. Atherosclerosis 2002;163(2):339-47.
15. D'Anna R, Baviera G, Corrado F, Cancellieri F, Crisafulli A, Squadrito F. The effect of the phytoestrogen genistein and hormone replacement therapy on homocysteine and C-reactive protein level in postmenopausal women. Acta Obstet Gynecol Scand 2005;84(5):474-7.
16. Gong DK, Liu BH, Tan XH. Genistein prevents cadmium-induced neurotoxic effects through its antioxidant mechanisms. Drug Res (Stuttg) 2015;65(2):65-9.
17. Zhang X, Tajima K, Kageyama K, Kyoi T. Irsogladine maleate suppresses indomethacin-induced elevation of proinflammatory cytokines and gastric injury in rats. World J Gastroenterol 2008;14(30):4784-90.
18. Rajashekhara N, Ashok BK, Sharma PP, Ravishankar B. The evaluation of anti-ulcerogenic effect of rhizome starch of two source plants of Tugaksheeree (*Curcuma angustifolia* Roxb. and *Maranta arundinacea* Linn.) on pyloric ligated rats. Ayu 2014;35(2):191-7.
19. Al Asmari AK, Al Omani S, Elfaki I, Tariq M, Al Malki A, Al Asmary S. Gastric antisecretory and antiulcer activity of bovine hemoglobin. World J Gastroenterol 2013;19(2):3291-9.
20. Bancroft J, Stevens A. In: Stevens A, editors. Theory and Practice of Histological Techniques. New York: Churchill Livingstone; 1982. p. 188-90.
21. Park JM, Han YM, Kangwan N, Lee SY, Jung MK, Kim EH, *et al.* S-allyl cysteine alleviates nonsteroidal anti-inflammatory drug-induced gastric mucosal damages by increasing cyclooxygenase-2 inhibition, heme oxygenase-1 induction, and histone deacetylation inhibition. J Gastroenterol Hepatol 2014;29 Suppl 4:80-92.
22. Sabiu S, Garuba T, Sunmonu TO, Sulyman AO, Ismail NO. Indomethacin-induced gastric ulceration in rats: Ameliorative roles of *Spondias mombin* and *Ficus exasperata*. Pharm Biol 2015;27:1-7.
23. Choi YJ, Kim N, Lee JY, Nam RH, Chang H, Seo JH, *et al.* Protective effects of garlic extract, PMK-S005, against nonsteroidal anti-inflammatory drugs-induced acute gastric damage in rats. Dig Dis Sci 2014;59(12):2927-34.
24. Moustafa YM, Khoder DM, El-Awady EE, Zaitone SA. Sildenafil citrate protects against gastric mucosal damage induced by indomethacin in rats. Eur Rev Med Pharmacol Sci 2013;17(2):179-88.
25. Albayrak A, Alp HH, Suleyman H. Investigation of antiulcer and antioxidant activity of moclobemide in rats. Eurasian J Med 2015;47(1):32-40.
26. Gomathy G, Venkatesan D, Palani S. Gastroprotective potentials of the ethanolic extract of *Mukia maderaspatana* against indomethacin-induced gastric ulcer in rats. Nat Prod Res 2015;29(22):2107-11.
27. Kaplan KA, Odabasoglu F, Halici Z, Halici M, Cadirci E, Atalay F, *et al.* Alpha-lipoic acid protects against indomethacin-induced gastric oxidative toxicity by modulating antioxidant system. J Food Sci 2012;77(11):H224-30.
28. Saha S, Sadhukhan P, Sil PC. Genistein: A Phytoestrogen with multifaceted therapeutic properties. Mini Rev Med Chem 2014.
29. Behloul N, Wu G. Genistein: A promising therapeutic agent for obesity and diabetes treatment. Eur J Pharmacol 2013;698(1-3):31-8.
30. Jeong JW, Lee HH, Han MH, Kim GY, Kim WJ, Choi YH. Anti-inflammatory effects of genistein via suppression of the toll-like receptor 4-mediated signaling pathway in lipopolysaccharide-stimulated BV2 microglia. Chem Biol Interact 2014;212:30-9.
31. Ganai AA, Khan AA, Malik ZA, Farooqi H. Genistein modulates the expression of NF-?B and MAPK (p-38 and ERK1/2), thereby attenuating d-Galactosamine induced fulminant hepatic failure in Wistar rats. Toxicol Appl Pharmacol 2015;283(2):139-46.
32. Mullane KM, Kraemer R, Smith B. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. J Pharmacol Methods 1985;14:157-67.
33. Seibel J, Molzberger AF, Hertrampf T, Laudenbach-Leschowski U, Diel P. Oral treatment with genistein reduces the expression of molecular and biochemical markers of inflammation in a rat model of chronic TNBS-induced colitis. Eur J Nutr 2009;48(4):213-20.
34. Wang R, Tu J, Zhang Q, Zhang X, Zhu Y, Ma W, *et al.* Genistein attenuates ischemic oxidative damage and behavioral deficits via eNOS/Nrf2/HO-1 signaling. Hippocampus 2013;23(7):634-47.
35. Javanbakht MH, Sadria R, Djalali M, Derakhshanian H, Hosseinzadeh P, Zarei M, *et al*. Soy protein and genistein improves renal antioxidant status in experimental nephrotic syndrome. Nefrologia 2014;34(4):483-90.
36. Ibrahim WH, Habib HM, Chow CK, Bruckner GG. Isoflavone-rich soy isolate reduces lipid peroxidation in mouse liver. Int J Vitam Nutr Res 2008;78(4-5):217-22.