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Research Article
Potential Antioxidant, Anti-Inflammatory and Gastroprotective Effect of Grape Seed Extract in Indomethacin-induced Gastric Ulcer in Rats

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Abstract

Background and Objective: Gastric ulcer is usually accompanied by an imbalance between the pro-inflammatory cytokines and the gastroprotective agents that save the lining of the stomach, the most important of which are antioxidants. This study aimed to explore the potential protective action of grape seed extract (GSE) against indomethacin (IND)-induced gastric ulcer and to compare the results with a standard antiulcer drug, pantozol (Panto). Furthermore, the underlying mechanism will be explored focusing on the oxidative stress and inflammation. **Materials and Methods:** Gastric ulcer was induced by a single oral dose of IND (30 mg kg^{-1}). Rats were pretreated with Panto (20 mg kg^{-1}), GSE (100 mg kg^{-1}) or both Panto+GSE once daily for 14 days before ulcer induction. **Results:** Gross evaluation of gastric mucosal lesions showed that Panto, GSE and Panto+GSE pretreatment reduced gastric lesions induced by IND. In addition, pretreatment with Panto, GSE and Panto+GSE before ulcer induction diminished ulceration of surface epithelium and maintained the normal histological structure of gastric mucosa. Ulcer index (UI), total gastric acidity and pH were significantly reduced in rats pretreated with Panto, GSE and Panto+GSE group. Pretreatment with GSE, Panto and GSE+Panto significantly decreased gastric mucosal oxidative stress (malondialdehyde, MDA), serum pro-inflammatory cytokines (tumor necrosis factor (TNF)-α and interleukin (IL)-6) compared with IND group. In these groups, a remarkable increase in the gastric tissues content of nitric oxide (NO) and prostaglandin (PG) E2 was also detected. Co-pretreatment with GSE+Panto showed a better ulcer healing capacity and compared favorably well with Panto results. **Conclusion:** These results concluded a gastroprotective effect of GSE against IND-induced gastric ulcer. This could be attributed to its antioxidant and anti-inflammatory actions. Furthermore, a combination of GSE and Panto provoked a better healing effect compared to GSE alone and Panto alone.

Key words: Grape seed extract, indomethacin, gastric ulcer, oxidative stress, ulcer index

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Globally, the stomach ulcer is a common digestive disease. There are many pathological factors that cause stomach ulcers such as *Helicobacter pylori*, non-steroidal anti-inflammatory drugs (NSAIDs) and stress[1-4]. Although the mechanism for gastric ulcers is complex, the incidence of the disease is usually accompanied by an imbalance between the protective agents of the stomach mucous membrane and the pro-inflammatory cytokines. Pro-inflammatory cytokines cause local inflammation of the mucosal lining of the stomach. With recurrent inflammation, the lining of the stomach is injured and the ulcer is formed. Furthermore, the lack of gastric secretion of protective factors such as antioxidants causes the stomach ulcer to occur and worsen[5,6]. Therefore, the strategy of treatment of gastric ulcers depends mainly on increasing the secretion of protective agents of the stomach mucosal membrane while at the same time inhibiting the secretion of pro-inflammatory cytokines[7].

Several studies have been conducted regarding the development of a wide spectrum of ulcer drugs, like proton pump inhibitors, antiacids, anticholinergics and histamine receptor antagonists[8-10]. However, most of these drugs are not always effective, have harmful toxicities and considerably expensive. Therefore, discovering natural agents, which are believed to be safe, effective and affordable is still essential for ulcer therapy[11,12].

Indomethacin (IND)-induced ulcer in rats is a widely used experimental model to assess the pathophysiology of NSAIDs and for screening of gastroprotective agents[13]. The mechanism of gastric ulcer developed with IND is due to its ability to inhibit the production of prostaglandins and to stimulate the formation of free radicals[14].

Grape seed extract (GSE) is a good source of the bioflavonoids compound, proanthocyanidin, which possesses a powerful antioxidant action exceeding that of vitamin E and vitamin C[15]. Proanthocyanidin exerted also a protective effect against free radicals-induced lipid peroxidation and DNA damage[16-18].

Because, the main mechanism beneath IND-induced gastric ulcer is free radical production and GSE was found to possess strong antioxidant properties, this study aimed to explore the potential protective action of GSE against IND-induced gastric ulcer and to compare the results with a standard antiulcer drug, pantozol (Panto). Furthermore, the underlying mechanism will be explored focusing on the oxidative stress and inflammation.

MATERIALS AND METHODS

Chemicals: The IND (Hikma Pharmaceuticals PLC, Amman, Jordan, provided as 25 mg/capsule) and Pantozol® (Pantoprazole Sodium sesquihydrate, Takeda GmbH, Konstanz, Germany as 20 mg/tablet). The required doses were calculated according to the weight of each animal. Other chemicals and reagents were purchased from Sigma-Aldrich (St Louis, MO, USA). This study was conducted in October, 2017 and the experimental protocol took about 7 months.

Grape seed extract (GSE): Grape (*Vitis vinifera*) seed extract (GSE) (standardized for 95% proanthocyanidins) was purchased from GNC ARMAL, Jeddah, Saudi Arabia. The GSE was available in the form of 100 mg capsules. GSE capsules were dissolved in distilled water and given orally at a dose of 100 mg kg^{-1}/day for 14 days[18].

Gastric ulcer induction: Gastric ulcer was induced in rats as described by Bhattacharya *et al.*[19]. The animals were fasted for 24 h before oral administration of a single dose of IND (30 mg kg^{-1}). Different degrees of gastric mucosal injuries were detected 4 h after IND administration.

Ethical approval: This study was approved by Deanship of Scientific Research Committee, King Abdulaziz University, reference no (G-708-253-38). The experiment was conducted at King Fahd Medical Research Center (KFMRC), KAU.

Experimental design and animal grouping: Forty-eight adult male albino Wistar rats weighing 190-210 g were used in this study. They were purchased from animal house of King Fahd Medical Research Centre (KFMRC), KAU, Jeddah, Saudi Arabia. During this study, the rats were kept under the rules of KFMRC ethical committee. After one week of acclimatization to the laboratory environment, the rats were randomized into the following 6 groups: Group I: (control), rats received distilled water orally. Group II: (GSE), rats orally received GSE (100 mg kg^{-1}) daily for 14 days. Group III: (IND) rats received a single dose of IND (30 mg kg^{-1}) orally. Group IV: (Panto+IND) rats in this group received Panto (20 mg kg^{-1})[20] daily for 14 days before IND. Group V: (GSE+IND) rats in this group received GSE (100 mg kg^{-1}) daily for 14 days before IND. Group VI: (GSE+Panto+IND) rats in this group received both GSE (100 mg kg^{-1}) and Panto (20 mg kg^{-1}) daily for 14 days before IND.

At the end of the experiment (4 h post IND injection) rats were sacrificed. The stomach was dissected out and cut along its greater curvature, then its content was evacuated into a centrifuge tube, diluted with distilled water and centrifuged at 12000 g for 10 min. Gastric pH and total gastric acidity were detected in the supernatant. Cleaned stomach was preserved in 0.1 M PSB and processed for macroscopic examination, homogenization and histopathological examination. Blood samples were collected, sera were separated and kept frozen until used for pro-inflammatory cytokines determination.

Determination of gastric pH: Gastric juice (1 mL) was diluted by distilled water (1 mL) in an aliquot to measure pH using pH meter[21].

Determination of total gastric acidity: Gastric fluid supernatant (1 mL) was diluted in a conical flask by distilled water (1 mL). Phenolphthalein indicator (2 drops) was added, then the mixture was titrated with 0.01 N NaOH till detection of a permanent pink color. The volume of 0.01 N NaOH (V NaOH) consumed was recorded. The total acidity (mEq L^{-1}) was calculated as following[21]:

$$\text{Acidity} = \text{V NaOH} \times N \times 100$$

where, N is normality.

Quantification of ulcer index and percentage inhibition of ulceration: Image Pro Express analyzer computer system was used to quantify the gastric ulcer index. The sum of gastric ulcer areas of all lesions for each stomach was used in the calculation of the ulcer area (mm^2). The total area of mucosa and the total area of ulcers area were calculated. Then, ulcer index and percentage inhibition of ulceration were calculated from the following equations[22]:

$$\text{Ulcer index (UI)} = \frac{\text{Total area of mucosal ulcers}}{\text{Total mucosal area}}$$

$$\text{Inhibition of ulceration (\%)} = \frac{\text{UI (IND group)-UI (Test group)}}{\text{UI (IND group)}} \times 100$$

Preparation of gastric mucosal homogenate: The gastric mucosa was homogenized in PBS (1:9) using a Teflon pestle (Ultra-Turrax, IKA: T25 digital, Germany) and centrifuged at 12000xg for 20 min at 4°C (Centurion, K280 R, UK). The supernatant was used for the estimation of biochemical parameters.

Measurement of antioxidant biomarkers and gastric protective factors in gastric mucosal homogenate supernatant: Oxidative stress biomarkers (malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT)), ulcer protective factors (nitric oxide (NO) and prostaglandin E2 (PGE2)). MDA, SOD, CAT, NO and PGE2 ELISA kits were obtained from MyBiosource, San Diego, California, USA.

Measurement of serum levels of pro-inflammatory cytokines: Pro-inflammatory cytokines (interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α)) were measured by using ELISA kits obtained from MyBiosource, San Diego, California, USA. The procedures were performed according to the manufacturer's protocols.

Preparation of gastric tissues for histopathological examination: The formaldehyde fixed stomach is paraffin-embedded, cut into sections, then stained with Hematoxylin-Eosin (H and E). The slides examined microscopically.

Statistical study: Ulcer inhibition was expressed in percentage. Results are reported as Mean±SE. Data were compared by one-way analysis of variance (ANOVA), followed by LSD, to determine the statistical significance of the difference using SPSS version 22. The $p<0.05$ indicate significance difference.

RESULTS

Effect of GSE on gastric mucosal lesions biomarkers (UI, percentage inhibition of ulceration, gastric pH and total gastric acidity): The gastric lesions biomarkers of the GSE group showed no significant difference compared with control group. In IND group, the subserosal layer of the glandular part of the anterior gastric wall showed a significant increase in UI compared with control group ($p<0.05$). On the other hand, in GSE+IND and Panto+IND groups a significant decrease ($p<0.05$) in UI was observed compared with IND group. Total gastric acidity decreased significantly while gastric pH increased significantly in both GSE+IND and Panto+IND groups compared with IND group ($p<0.05$). In GSE+Panto+IND group there was a significant increase in gastric pH with a significant decrease in both UI and total gastric acidity compared with IND group ($p<0.05$). Furthermore, in GSE+Panto+IND group there was a significant decrease in both the total gastric acidity and the UI compared with GSE+IND group and Panto+IND group ($p<0.05$) (Table 1).

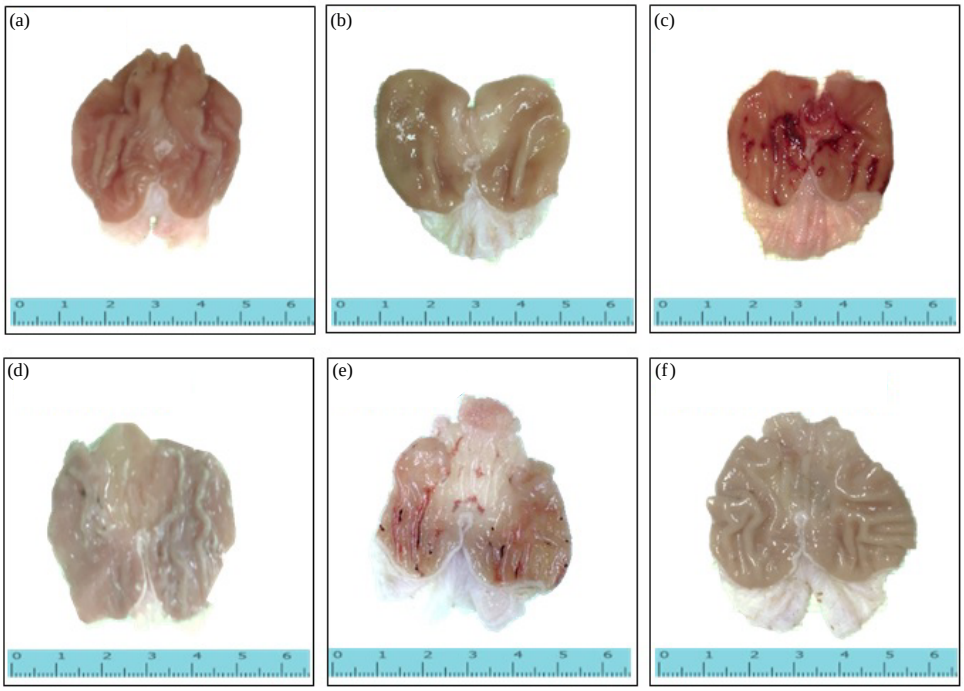

Fig. 1(a-f): Gross appearance of (a) Control, (b) GSE, (c) IND, (d) Panto+IND, (e) GSE+IND and (f) GSE+panto+IND, gastric mucosa (Control) and (GSE) showing the pink glandular part (normal rugae), mucosa of IND rats seems to be hyperaemic with obviously darkish patches showing macroscopic mucosal areas of different sizes and color, pretreatment with Panto or GSE showing nearly normal mucosae with tiny ulcers, while pre-treatment of Panto+GSE has nearly normal mucosa

Table 1: Effect of IND, GSE and/or Panto on ulcer index (UI), percentage inhibition of ulceration, gastric pH and total gastric acidity

Experimental groups	UI (mm^2)	Ulcer inhibition rate (%)	Gastric pH	Total gastric acidity
Control	-	-	3.43±0.189	56.24±3.09
GSE	-	-	3.24±0.182	47.04±1.60
IND	39.69±3.83[a]	-	2.32±0.188[a]	185.54±5.08[a]
Panto+IND	20.31±2.42[b]	48.83	3.75±0.171[b]	89.23±5.58[b]
GSE+IND	29.25±2.25[b]	26.30	3.80±0.184[b]	100.26±5.82[b]
GSE+Panto+IND	7.63±0.74[b,c,d]	80.78	3.96±0.142[b]	63.39±6.68[b,c,d]

Data are represented as Mean±SE (n = 8), [a]Significant vs. control, [b]Significant vs. IND, [c]Significant vs. Panto+IND, [d]Significant vs. GSE+IND ($p \leq 0.05$)

Gross evaluation of gastric mucosal lesions: Macroscopic appearances of gastric tissues were shown in Fig. 1. Control and GSE groups showed normal glandular gastric with regular rugae. The IND-induced extensive and detectable hemorrhagic lesions in the gastric mucosa. Both Panto and GSE pretreatment reduced gastric lesions induced by IND; however, co-pretreatment with both Panto and GSE was the most protective of gastric lesions, the reduction of ulcer formation was significant as compared to that observed in either the Panto or GSE alone pretreatment groups.

Gastric mucosa histopathological changes: The histopathological examination of the fundic mucosa of different groups was shown in Fig. 2. Control rats showing crowded normal gastric mucosal crypts extending in lamina propria. The mucus-secreting surface columnar epithelial cells were intact. The upper parts of the glands had many acidophilic parietal cells with central rounded nuclei. The lamina propria is rich in blood vessels and it is separated from the submucosa by muscularis mucosa (Fig. 2a). The gastric mucosa of GSE group is as normal as the control group with an apparent increase in mucous-secreting cells, prominent dilation in the gastric pits and lumen of gastric glands (Fig. 2b). In comparison, in IND group there are different grades of mucosal injuries. Damaged glandular areas showed severe destruction of the surface epithelium and necrotic lesions as well as the severe edema of submucosa layer. In addition, the lower parts of the gastric glands showed prominent cytoplasmic vacuolations. Mononuclear cellular infiltration is also noticed in lamina propria (Fig. 2c, d). Pretreatment with

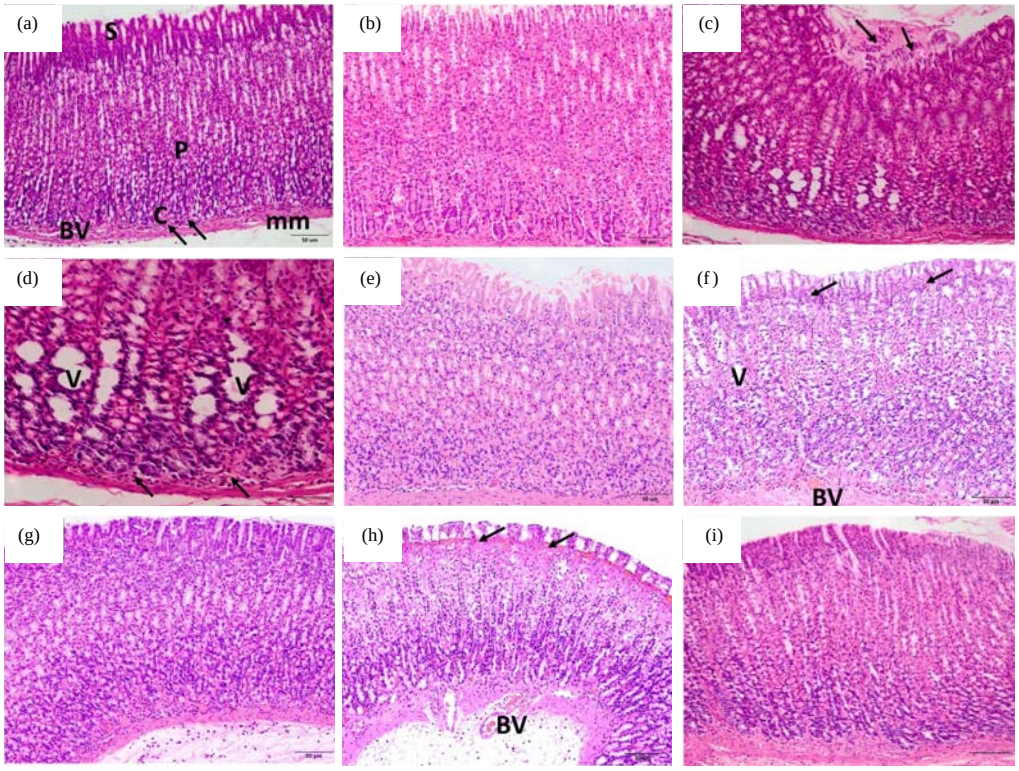

Fig. 2(a-i): Photomicrography illustrating H and E-stained sections of gastric mucosa of (a) Control, (b) GSE, (c, d) IND, (e, f) Panto+IND, (g, h) GSE+IND and (i) GSE+Panto+IND groups. Arrows in photo a showed deep mucosal layer. Arrows in photo c represented exfoliated necrotic superficial epithelium in ulcer area. Arrows in photo d represented mononuclear cellular infiltrations in the deep mucosal layer. Arrows in photo f and h represented normal superficial epithelium layer

BV: Blood vessels, C: Chief cells, P: Parietal cells, mm: Muscularis mucosa, S: Superficial epithelium layer, V: Vacuolations

Table 2: Effect of IND, GSE and/or Panto on gastric mucosal PGE2 and NO

Experimental groups	PGE2 (ng g^{-1} tissue)	NO (μ mol g^{-1} tissue)
Control	178.44±1.27	0.492±0.05
GSE	187.63±3.87	0.514±0.01
IND	101.43±1.32[a]	0.161±0.007[a]
Panto+IND	131.94±4.24[b]	0.279±0.007[b]
GSE+IND	124.13±3.93[b]	0.249±0.018[b]
GSE+Panto+IND	168.06±4.08[b,c,d]	0.425±0.047[b,c,d]

Data are represented as Mean±SE (n = 8), [a]Significant vs. control, [b]Significant vs. IND, [c]Significant vs. Panto+IND, [d]Significant vs. GSE+IND ($p \leq 0.05$)

Panto before ulcer induction diminished ulceration of surface epithelium and maintained the normal histological structure. In some sections, restricted areas of mucosal hyperemia, leucocytic infiltration and congested dilated blood vessels in the lamina propria are prominent (Fig. 2e, f). Animals pretreated with GSE have a markedly better protection of their gastric mucosa. The gastric glands appeared mostly near normal except for few mucosal areas of superficial ulceration and hyperemia in between intact mucosa (Fig. 2g, h). The pretreatment of rats with both Panto and GSE group preserved intact mucosa nearly having the normal histological structure similar to the control group. Therefore, it recorded more potent protective effect than treatment with each one of them alone (Fig. 2i).

Effect of GSE on ulcer protective factors: In IND group, there was a significant decrease in the PGE2 and NO compared with the control group ($p<0.05$). The pretreatment of rats with either GSE or Panto induced a significant increase in the gastric mucosa PGE2 and NO levels as compared to IND group ($p<0.05$). In GSE+Panto+IND group, there was a significant increase in the gastric PGE2 and NO compared with IND group ($p<0.05$). Furthermore, in GSE+Panto+IND group there was a significant increase in gastric PGE2 and NO compared with GSE+IND group and Panto+IND group ($p<0.05$) (Table 2).

Effect of GSE on oxidative stress biomarkers: In the IND group, there was a significant decrease in the gastric mucosal CAT and SOD activities with a significant increase in MDA level as compared to control values ($p<0.05$). The pretreatment of

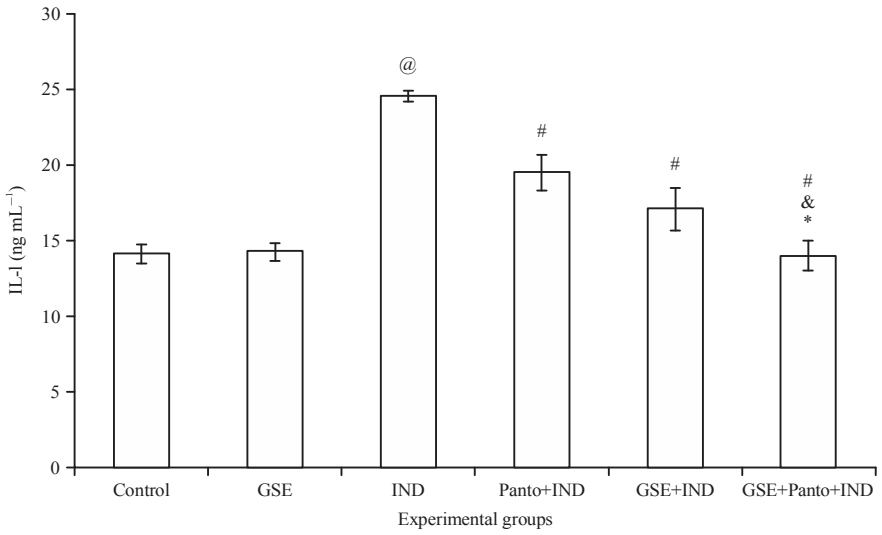

Fig. 3: Effect of IND, GSE and/or Panto on serum IL-6

Data are represented as Mean±SE (n = 8), @Significant vs. control, #Significant vs. IND, &Significant vs. Panto+IND, *Significant vs. GSE+IND ($p \leq 0.05$)

Table 3: Effect of IND, GSE and/or Panto on gastric mucosal CAT, SOD and MDA

Experimental groups	CAT (μ mol g^{-1} tissue)	SOD (μmol g^{-1} tissue)	MDA (μmol g^{-1} tissue)
Control	356.11±9.09	71.68±2.69	0.260±0.011
GSE	382.62±12.68	79.64±4.55	0.231±0.011
IND	202.94±3.11[a]	45.69±1.71[a]	0.943±0.067[a]
Panto+IND	269.86±8.15[b]	57.98±2.79[b]	0.411±0.053[b]
GSE+IND	306.54±24.56[b]	65.14±2.59[b]	0.290±0.006[b]
GSE+Panto+IND	341.77±9.5[b,c]	70.59±2.71[b,c]	0.264±0.008[b,c]

MDA: Malondialdehyde, CAT: catalase, SOD: Superoxide dismutase, Data are represented as mean±SE (n = 8). [a]Significant versus control, [b]Significant versus IND, [c]Significant versus Panto+IND and [d]Significant versus GSE+IND ($p \leq 0.05$)

rats with either GSE or Panto resulted in a significant increase in the gastric mucosa CAT and SOD enzyme activities with a significant decrease in the gastric mucosa MDA as compared with IND group ($p<0.05$). In GSE+Panto+IND group, oral pretreatment with GSE and Panto showed more effective protection than each one alone, as it produced reduction of MDA level and elevation in CAT and SOD enzyme activities greater than either GSE or Panto alone. Furthermore, in GSE+Panto+IND group there was a significant increase in gastric CAT and SOD with a significant decrease in MDA compared to Panto+IND group ($p<0.05$). Conversely, in GSE+Panto+IND group there was a non-significant difference in gastric CAT, SOD and MDA compared with GSE+IND group (Table 3).

Effect of GSE on serum pro-inflammatory cytokines: In IND group, there was a significant increase in the levels of IL-6 and TNF-α versus with control group ($p<0.05$). The pretreatment of either GSE, Panto or GSE+Panto significantly decreased the levels of IL-6 and TNF-α versus IND group ($p<0.05$). Oral pretreatment with both GSE and Panto is more effective than either GSE or Panto alone. Furthermore, in GSE+Panto+IND group there was a significant decrease in serum IL-6 compared to Panto+IND group ($p<0.05$) and GSE+IND group ($p<0.05$). In addition, in GSE+Panto+IND group there was a significant decrease in serum TNF-α compared to Panto+IND group ($p<0.05$) (Fig. 3, 4).

DISCUSSION

This experimental work aimed to investigate whether GSE can protect against IND-induced gastric ulcer compared to the reference anti-ulcer drug, Panto. In addition, this study evaluated the gastroprotective effect of a combination of GSE and Panto against IND-induced gastric ulcer. The results of this work showed that pretreatment with GSE, Panto and GSE+Panto significantly decreased UI, total gastric acidity and increased gastric pH compared to IND group. Furthermore, the combination of GSE and Panto showed superior activity against IND-induced changes in UI and total gastric acidity

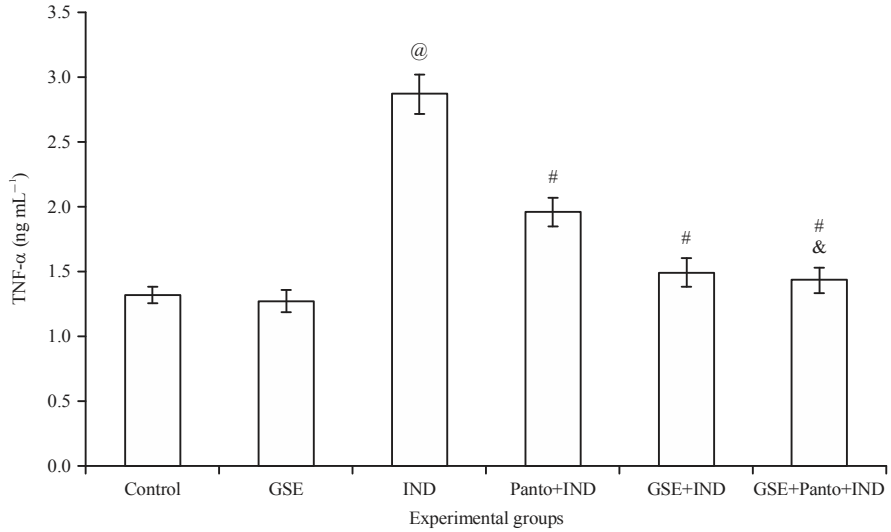

Fig. 4: Effect of IND, GSE and/or Panto on serum TNF-α

Data are represented as Mean±SE (n = 8), @Significant vs. control, #Significant vs. IND, &Significant vs. Panto+IND, *Significant vs. GSE+IND ($p \leq 0.05$)

compared to either GSE or Panto alone. The gross appearance and histopathologic results of this study clearly showed a significant gastroprotective effect of GSE, Panto and GSE+Panto against IND-induced gastric ulcer. These results are similar to previous findings which documented the gastroprotective effect of GSE in many experimental models of gastric and intestinal mucosal ulcer[23-25]. Low pH value has been linked to causing ulcers and destroying the stomach in animal models[26]. This lesion occurs through internal defeats such as pepsin and oxidative stress and external factors such as drugs and chemicals that help the damage of gastric mucosal epithelium.

The macroscopic and histopathological results of present study demonstrated prominent mucosal injuries, hemorrhagic lesions and cytoplasmic vacuolations following IND treatment. This could be attributed to the increased formation of oxidative stress measures[27]. It has previously been discovered that a decrease in antioxidant enzymes in the stomachs induces gastric ulceration[28]. Free radicals initiate MDA which has a major role in the toxicity mechanism of IND[29]. The damage in gastric ulcers involves an increased level of MDA, which if not scavenged by antioxidant enzymes may lead to an increase in the accumulation of MDA that cause severe tissue damage[27,30]. Cells and tissues are protected from damage if there is a balance between the formation of free radicals and its scavenging mechanisms. An unevenness between them caused oxidative stress and disturbs cellular functions[27].

In current study, GSE induced significant antioxidant effects as markedly by the increase in gastric activity of both CAT and SOD and a significant decrease in MDA level compared with IND. This could be attributed to GSE active constituents which include bioflavonoid and proanthocyanidin which play a vital role as a scavenger of free radicals and performs powerful antioxidant function[24,31].

The NO is an endogenous defensive agent for gastric cells[32]. The PG, is the main molecule that arouses the complex array of ulcer healing mechanism, is synthesized in the mucosal cells by cyclo-oxygenase enzymes, stimulates the secretion of bicarbonate and mucus, regulates mucosal turnover and repair and maintains mucosal blood flow[33]. A decrease in PGE2 and NO levels have been related to disruption of gastroprotection and elevated gastric acid secretion, which has a major role in the etiology of mucosal ulceration[34,35]. Present results showed that, IND induced a significant decrease in PGE2 and NO with a significant increase in cytokines IL-6 and TNF-α compared with control group. Interestingly, pretreatment with GSE or Panto significantly ameliorated these parameters. Like our results, previous studies reported that IND induces gastric lesions through a number of mechanisms which includes interfering with PGE2 synthesis, increasing acid secretion and increased IL-6 formation[36,37]. On the other hand, GSE stimulates the production of PGE2, which enhances mucus secretion resulting in protection of stomach against IND-induce ulcer[38,39].

Cytokines play a pivotal role in the mechanism of inflammation. During the ulcer formation, TNF-α causes an increase in the flow of inflammatory cells such as neutrophil to the gastric tissue, which increases the severity of the ulcers and delays the healing process as it stabilizes the inflammatory process[40-42]. The IL-6 also stimulates the transmission of inflammatory cells such as macrophages and lymphocytes to the lesions sites and also increases free radicals and oxidative stress, as well as increases the lysosomal enzymes responsible for tissues death during ulcers[43]. Similarly, Li *et al.*[44] reported that GSE decreased the production of pro-inflammatory cytokines in rats' colon and in pylorus ligation model. Consequently, another possible mechanism of the gastroprotective effect of GSE may be due to the inhibition of pro-inflammatory cytokines IL6 and TNF-α, which are involved in the production of acute inflammation and gastric mucosal injury[45,46].

Co-pretreatment with GSE and Panto showed a better ulcer healing capacity and compared favorably well with the reference drug used. Besides antioxidant and anti-inflammatory action of GSE that enhances antioxidant status, protects the mucus layer and stops the development of the ulcer, Panto is a proton pump inhibitor. This, in turn, has modulated cells in the mucosal lining of the gastric and stimulated gastric healing of the ulcerated areas of the mucosal epithelia and shielded the gastric membrane, thus abrogating the disastrous influence of IND in the ulcerated rats.

CONCLUSION

This study results showed a gastroprotective effect of GSE against IND-induced stomach ulcer. This could be attributed to its antioxidant and anti-inflammatory actions. Furthermore, a combination of GSE and Panto provoked a better healing effect compared to GSE alone and Panto alone.

SIGNIFICANCE STATEMENT

The results of this study showed for the first time that a combination of GSE and Panto provoked a better healing effect compared to GSE alone and Panto alone. This finding will be beneficial to many patients suffering from the NSAIDs-induced gastric ulcer. Also, it opens the search field in front of researchers to search the protective effect of the GSE extract combined with Panto in human beings.

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