Protective Effect of Pomegranate Peels Extracts Against Stomach Peptic-Ulcer Induced By Brexin In Albino Rats

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Research Article

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Abstract

Background: Pomegranate peel extract (PPE) is known to possess bioactive compounds such as phenolics and flavonoids, considered among the more potent antioxidants and anti-inflammatory sources.

Aims: This study was designed to evaluate PPE activity's protective effect as a natural therapeutic against peptic ulcers induced by Brexin.

Methods: 40 rats were divided into four groups: Control group: ten rats received normal saline treatment; Brexit group: ten rats received a single oral dose of Brexin to induce the stomach ulcer; Antodine group: ten rats received Antodine (50 mg/kg) as a commercial drug for the peptic ulcer treatment once for two weeks following a peptic ulcer; Pomegranate group: ten rats of the group received PPE (43 mg/kg) treatments. Histological, histochemical, immunohistochemical techniques were used to detect histopathological damages in stomach rats in all groups.

Results: The histopathological results showed that PPE treatments following Brexin-induced peptic ulcer ameliorated histological degenerative changes in the gastric glandular. Chief and surface mucous cells that are lining gastric mucosa were regained when compared with the other groups. The histochemical results showed that PPE treatment following ulcer provided an improvement in the secretion and distribution of the polysaccharides in the epithelial cells when compared with the other groups. Also, immunohistochemical results indicated a significant decrease in immunoreactivity of cytokeratin-20, cyclooxygenase-2, and proliferating cell nuclear antigen (PCNA) in epithelial cells of rats in ulcer-model when compared with the other groups.

Conclusion: PPE revealed its antiulcer activity and is recommended as a natural remedy against gastric mucosal injury induced by Brexin.

Introduction

Peptic ulcers are considered the most common gastrointestinal diseases. A gastric ulcer is an interruption of the gastric mucosa's continuity that extends towards the muscularis mucosa and may reach deeper into the submucosa (Brooks 1985). It can cause major complications such as bleeding, perforation, and potentially death if associated with decompensation of coexisting medical conditions (Hernández-Díaz &Rodríguez 2002).

Anti-inflammatory drugs, including nonsteroidal anti-inflammatory drugs (NSAIDs), are considered essential analgesics for treating severe and acute pain (Moote 1992). Many studies have proven that NSAIDs drugs such as Brexin cause gastric ulcers, ulcer perforation, gastric and duodenal bleeding, and ulcer death (Avila et al. 1996a, Russell 2001). In contrast, a lot of the medication used commonly to treat peptic ulcer diseases includes prostaglandins analogs, histamine receptor antagonists, proton pump inhibitors, and cytoprotective agents designed to treat peptic ulcers (Jain et al. 2007).
H2 receptor antagonists, also called H2 blockers, are second-generation histamine H2-receptor antagonists that reduce stomach acid produced by the gastric mucosa (Ichikawa et al. 2009). H2 receptor antagonists commercially are presented under different names such as Famotidine, Nizatidine, and Antodine (Al-Omar & Al-Mohizea 2009, Schunack 1989). They are specific to inhibit and/or treat peptic ulcers and gastroesophageal reflux disease (Nash et al. 1994). Several studies have indicated the H2 receptor antagonist’s efficacy depends on their ability to prevent gastric acid production (Goldstein et al. 2006b). In this regard, H2 receptor antagonist has been associated with some histopathological alterations in gastric mucosal cells in the experimental animals (Kobayashi et al. 2000).

Medical plants have important pharmacological properties attributed to their antioxidant and anti-inflammatory potency (Adebajo et al. 2009). Pomegranate peels are considered the rich part of this fruit, which possesses higher polyphenols, such as gallic acid (El-Hamamsy & El-khamissi 2020). Pomegranate peel extracts are widely used to treat several diseases due to their polyphenols’ richness. The polyphenols play a powerful role against anti-inflammatory and antioxidant activity (Qabaha et al. 2019). Besides, pomegranate and its derivatives have positively demonstrated effects against several diseases (Viuda-Martos et al. 2010). Several natural products showed that pomegranate possesses anti-ulcerogenic activity by their predominant effects on mucosal defensive factors. Pomegranate peels are rich sources of tannins, flavonoids, polyphenols, and some anthocyanins as delphinidins and cyanidins (Abid et al. 2017). Pomegranate peels were found to have a protective effect against neuroinflammation (DaSilva et al. 2019), hepatotoxicity (Ahmad et al. 2016), renal damages (Kandeil et al. 2019), cardiovascular dysfunction (Wang et al. 2018a), metabolic syndromes (Hou et al. 2019), and autoimmune diseases (Wang et al. 2018b). This study aimed to investigate the potential protective role of pomegranate peel extract against gastric ulcers induced by Brexin.

**Materials And Methods**

**Drugs**

Brexin (each tablet contains 20 mg/kg piroxicam-beta-cyclodextrin) was purchased from Chiesi Company for pharmaceuticals and chemical industries, Cairo, Egypt. Antodine (each tablet contains 20 mg/kg famtodine) was purchased from Amoun company for pharmaceuticals, Cairo, Egypt.

**Plant extraction and high-performance liquid chromatography (HPLC) analysis**

Pomegranate fruits were purchased from the local market (Helwan, Cairo, Egypt). The peels of the pomegranate were removed and left to dry at room temperature. The peels were grounded to powder. Ten grams of pomegranate peel powder was extracted using 100 ml of ethanol using HPLC as described by (Saeed et al. 2020). The extracts were filtered and then centrifuged (4000 rpm) for 15 minutes and kept in the refrigerator until use. Total phenolics were defined in the extracts as described by (Singleton & Rossi 1965). While total flavonoids were determined as described by (Matyushchenko & Stepanova 2003).

**Induction of gastric ulcer**
In this study, the gastric ulcer was induced in rats by a single oral dose of Brexin (5 mg/kg) according to the described method by (Avila et al. 1996a).

**Animals and experimental design**

Forty albino rats (weight, 160-165 gm) were obtained from the animal’s house of Cairo University, Cairo, Egypt. Rats were housed in cages and offered water and food for one week for adaptation before starting the experiment. The animals were divided into four groups, as follows:

Control group: 10 rats received normal saline for two weeks.

Brexin group: 10 rats were administered a single oral dose of Brexin to induce the stomach ulcer (Avila et al. 1996b).

Antodine group: 10 rats received 50 mg/kg/body weight of Antodine oral suspension as a commercial drug once for two weeks to treat peptic ulcers following Brexin-induced peptic ulcers (Alkushi & Elsawy 2017).

Pomegranate group: 10 rats received PPE orally (500 mg/kg) once daily for two weeks following after Brexin-induced peptic ulcer (Abbasi et al. 2015). All animals were killed by cervical dislocation after 24 hours after the last dosage.

**Sample collection**

The animals were killed by cervical dislocation 24 hours after the last dosage. The stomach was quickly dissected, and about 0.5 cm of the stomach tissue was cut, cleaned, washed, and fixed for histopathological, electron microscope, and immunohistochemical studies.

**Histological and histochemical studies**

The sample was fixed in 4% formaldehyde (24 hours) for histological and histochemical studies. The samples were then dehydrated and impregnated in paraplast paraffin wax and then cut into sections of 4-μm. For the histological examination, the sections were stained by hematoxylin and eosin (H&E) (Bancroft & Gamble 2008) to assess gastric injury using a light microscope (Nikon Eclipse E200-LED, Tokyo, Japan). The histochemical study was performed for all groups using the method as described by (Cardiff et al. 2014). The sections were stained by the periodic acid-Schiff’s (PAS) method to qualitatively detect mucin polysaccharides in the epithelial cells lining the stomach. Mucopolysaccharides were evaluated using semi-quantitative scales to estimate staining intensity and the proportion of positive cells (Milosevic et al. 2015).

**Transmission and scanning electron microscope studies**

For the transmission electron microscope study, a small piece of stomach tissue (1mm) was fixed in phosphate-buffered saline (PBS) solution (containing 2.5% glutaraldehyde and 1% paraformaldehyde) for
two hours. Subsequently, samples were stored in 1% osmium tetroxide with 0.1 M sodium cacodylate for one hour. The samples were dehydrated in graded ethanol and embedded in resin. The blocks were cut (thickness 60–80 nm) and mounted on copper grids. The sections were stained with 2% aqueous uranyl acetate for five minutes and re-stained with 0.1 M lead citrate for five minutes and left to dry at room temperature. The grids were examined using a transmission electron microscope (TEM) (JEOL-JEM-1010, Al-Azhar University). For the scanning electron microscope (SEM) study, the samples were washed in PBS solution and then fixed in cacodylate for SEM based on the method was described by (Bancroft & Gamble, 2008). The sections were examined by Jeol JM6700 F scanning microscope (Tokyo, Japan).

**Immunohistochemical study**

Ten sections (4-μm thickness) from each group were incubated with the primary antibodies (cytokeratin-20 (1:200) monoclonal antibody (SA35-03, catalog, MA5-31979, RRID AB-2809273), cyclooxygenase-2 (1:100) monoclonal antibody (catalog, MA5-14568, RRID AB-10984436)) from Thermo Fisher Scientific, and rabbit polyclonal anti-proliferating cell nuclear antigen (PCNA) (1:50) (catalog, HPA030523-100UL, Merck KGaA, Darmstadt, Germany) for two hours as described by (Jensen 2008). Subsequently, sections re-incubated with the secondary antibody using the immunoperoxidase technique as described by the Sigma company (Sigma-Aldrich, USA). The slides were examined and imaged using a light microscope (Nikon Eclipse E200-LED, Tokyo, Japan). Scoring and analysis of immunostaining cells in this study were performed as described by (Albrakati 2020).

**Statistical analyses**

Data were expressed as mean ± SD. Significant differences were determined by using one way ANOVA and SPSS. Duncan's test and post hoc test were used to detect the statistical significance between-group comparison. p ≤ 0.05 is considered as a significant level.

**Results**

**HPLC analyses of PPE**

Polyphenol and flavonoid compounds of the PPE are illustrated in Fig. 1. PPE's HPLC profile demonstrates five peaks at different retention times, in the range from 10.853 to 30.728 min. The ultraviolet-visible spectral data show PPE has bands at 280 nm, characteristic for polyphenols and flavonoids compounds. Coumaric acid, caffeic acid, ellagic acid, cinnamic acid, and quinic acid may also be present.

**Histology results**

Examination of control rats showed normal histological structures of the epithelial cells, which line the gastric mucosa layer of the stomach body, as seen in Fig. 2A. In contrast, the ulcer-model rats' group's examination revealed degenerating parietal cells in the isthmus region accompanied by pyknosis. The microscopic study of rats in this group also showed chief cells' degeneration in the basa region
accompanied by a karyolitic nucleus, as seen in Fig. 2B. On the other hand, the examination of rats treated with Antodine, following Brexin-induced peptic ulcer, show a slight improvement of histopathological alteration of the epithelial cells in the mucosa layer (Fig. 2C). The microscopic examination of the rat group treated with pomegranate peels, following Brexin-induced peptic ulcer, shows a good improvement in the recovery of histopathological alteration of the epithelial cells of gastric glandular cells, chief, and surface mucous cells, as seen the Fig. 2D.

**Electron microscopic examination results**

**TEM results**

The TEM examination of chief cells from the stomach's gastric mucosal layer of rats in the control group appeared regular with an intact nucleus, mitochondria, and free ribosomes. Rough endoplasmic reticulum (rER) was seen intact in the cytoplasm with apical zymogenic secretory granules (Fig. 3A). In contrast, TEM examination of the gastric glandular cells of rats in the ulcer-model showed irregular nucleus membrane, with mitochondria degeneration and vacuolated zymogenic secretory granules, as seen in Fig. 3B. On the other hand, the examination of chief cells of rats treated with Antodine, following Brexin-induced peptic ulcer, appeared with a regular nucleus, few granules, and well-developed rER (Fig. 3C). TEM examination of the gastric glandular cells of rats in the pomegranate group showed improved recovery of chief cells with an intact nucleus and zymogen granules (Fig. 3D).

**SEM results**

Scanning examination of a control rat stomach sample revealed normal folds of gastric mucosa with regular gastric pits (Fig. 4A). Analysis of rats in the ulcer-model showed degeneration surface of the gastric mucosa (Fig. 4B). In contrast, the examination of treated rats with Antodine showed a slightly amorphous gastric mucosa on the surface (Fig. 4C). The treated rats with PPE analysis showed a mucus patches' accumulation on the gastric lining and narrowing of the gastric pits (Fig. 4D).

**Histochemistry results**

The histochemical examination result of rats in the control group showed a heavy PAS reaction of the surface mucous and mucous neck cells in the gastric mucosa layer (Fig. 5A). On the contrary, the histochemical examination of rats in the peptic-ulcer group illustrated a weak PAS reaction of the epithelial cell in the mucosa regain (Fig. 5B). The study of the treated rats with Antodine showed moderate PAS reaction of the epithelial cell in the mucosa region, as seen in Fig. 5C. In contrast, the examination of rats in the pomegranate group showed an improved PAS reaction of the epithelial cell in the mucosa regain (Fig. 5D).

The histochemical analysis results showed a significant decrease (p<0.05) of the polysaccharides distribution among gastric mucosa layers in the ulcer-model group compared to control, Antodine, and/or to pomegranate groups. The results also showed a non-significant polysaccharides distribution among
gastric mucosa layers when compared to Antodine and/or pomegranate groups with the control group (Fig. 9A).

**Immunohistochemistry results**

**Cytokeratin-20**

Immunohistochemical examination of cytokeratin-20 in the gastric mucosa layer's epithelial cells showed a weak expression for the control group rats, an intensive expression for the ulcer-model group, and moderate expression for the Antodine and pomegranate groups, as seen in Figs. 6A, 6B, 6C, and 6D, respectively.

The immunoreactivity analysis results showed a significant decrease (p<0.05) in the immunoreactivity of cytokeratin-20 in the epithelial cells of the gastric mucosa layer of rats in the ulcer-model group as compared to the control group, Antodine group, and/or to pomegranate groups. By contrast, the immunohistochemical analysis results showed a non-significant change in immunoreactivity of cytokeratin-20 in the epithelial cells of gastric mucosa layer in rats of Antodine and/or in pomegranate groups, as compared to the control group (Fig. 9B).

**Cyclooxygenase-2**

Immunohistochemical examination of cyclooxygenase-2 in the gastric mucosa layer's epithelial cells showed a mild expression for the control group samples, an intensive expression for the ulcer-model group, and a moderate expression for both Antodine and pomegranate groups, as seen in Figs. 7A, 7B, 7C, and 7D, respectively.

The immunohistochemical analysis results showed a significant increase (p<0.05) in the immunoreactivity of cyclooxygenase-2 for rats in the ulcer-model group compared to the control, Antodine, and/or to pomegranate groups. The immunohistochemical analysis results showed a non-significant change in the immunoreactivity of cyclooxygenase-2 in the gastric mucosa layer’s epithelial cells for rats in the Antodine and/or pomegranate groups, as compared to the control group (Fig. 9C).

**Proliferating cell nuclear antigen (PCNA)**

Immunohistochemical examination of the PCNA in the epithelial cells of the gastric mucosa layer showed a moderate expression for the control group rats, an intensive expression for the ulcer-model group rats, a weak expression for the Antodine group rats, and a moderate expression for the pomegranate group rats, as seen in the Figs. 8A, 8B, 8C, and 8D, respectively.

The immunohistochemical analysis results showed a significant increase (p<0.05) in the PCNA immunoreactivity of the gastric mucosal layer's epithelial cells for the ulcer-model group rats compared to the control, Antodine, and/or pomegranate groups. On the other hand, the immunohistochemical analysis
results showed a non-significant PCNA increase in the gastric mucosa layer's epithelial cells for rats in the Antodine and/or pomegranate groups as compared to the control group (Fig. 9D).

Discussion

Gastric ulcer is correlated in the literature with the dosage and period of NSAIDs drug exposure (Drini 2017, Goldstein & Cryer 2015). In the current study, the pomegranate peel extract's potential protective effect was investigated against gastric mucosal injury induced by Brexin.

The histopathological results in the current study showed that administration of Brexin induced several histological alterations in glandular and surface mucous cells of the gastric mucosal layer. The examination exhibited pyknosis and vacuolation among parietal cells in the isthmus region of the gastric mucosa layer. These results agree with previous histological findings reported by (Alazzouni et al. 2020), Avila et al. (1996a), (Sabiu et al. 2015). The NSAIDs induce histological changes in the gastric tissue by inhibiting prostaglandin production, which leads to increased acid levels (Beck et al. 2000). These events cause a decreased cytoprotective mucus formation and therefore induce gastric ulcers. In this regard, it has been reported that indomethacin administration could lead to accumulation of lipid peroxidation in the gastric tissue, and hence could cause peptic-ulcer (Adhikary et al. 2011).

Several studies have reported that ultrastructural damages in the gastric cells induced by anti-inflammatory have resulted in degenerative mitochondria that cause the release of cytochrome C. Consequently, activate reactive oxygen species (ROS) and then cellular apoptosis (Nagano et al. 2005). Brexin, an enolic acid-derived NSAID, induces gastric ulcerations by suppressing gastric prostaglandin synthesis leading to increased acid production and decreased cytoprotective mucus formation in agreement with the reports by Musumba et al. (2009). The injury effects of Brexin on the glandular cells mucus have been discussed in several studies (Salvatella et al. 2004). Brexin alters the cell membrane permeability and the mucus' nature, allowing back diffusion of hydrogen ions. Subsequently, it acts to degenerate the mitochondria in the chief cells and the microvilli (Avila et al. 1996a, Schoen & Vender 1989).

Our electron microscopic results for the ulcer-model group rats showed that Brexin caused several ultrastructural changes in the mucous cell organelles, including an irregularity in the nucleus membrane, mitochondria degeneration, and vacuolated zymogenic secretory granules. These results are in agreement with Alazzouni et al. (2020), who demonstrated that a single oral dose of Brexin in rats caused degeneration in surface mucous cells with an irregular pyknotic nucleus, fragmented rER, and mitochondria. Also, our results are in agreement with Halter et al. (2001), who demonstrated that treatment with aspirin in humans caused gastro mucosal damage, which resulted in a severe intramucosal petechial hemorrhage and erosions.

The histochemical results for the ulcer-model rats in the present study showed the ulcerative effect of Brexin on the gastric mucosa. Our work showed a significant decrease in the polysaccharides secretion for the ulcer-model rats compared with the other groups (Antodine, pomegranate, and control). This result
is in agreement with Alazzouni et al. (2020), who reported a noticeable decrease in the mucus polysaccharides distribution at the mucosal lining of rats due to the treatment with a single oral dose of Brexin as NSAIDs drug. Also, this finding agrees with the results by Mahmoud and Abd El-Ghffar (2019). They showed a weak distribution of polysaccharides at the surface of the epithelial cells lining of the gastric mucosa, caused by the treatment with aspirin as NSAIDs drug in mice.

The increased immunoreactivity of cytokeratin-20 is considered a marker for inflammation and oxidative stress that promotes cytoskeleton damage in the epithelial cells lining of the gastric mucosa (Todorovic et al. 2006). So, to provide more evidence on the ulcerative effect of the Brexin on gastric mucosa, cytokeratin-20 was measured in all the groups. Our immunohistochemical results showed a significant positive immunoreactivity of cytokeratin-20 in the vast majority of epithelial cells lining of the gastric mucosa in the ulcer model rats group. A similar result was reported by Alazzouni et al. (2020) who showed a positive immunoreactivity of cytokeratin-20 in the epithelial cells of the mucosal lining of rats following the treatment with a single oral dose of Brexin.

Prostaglandins play an essential role in preserving the gastric mucosa against the NSAIDs drug's ulcerative effect by an increase in blood flow and production of mucus and bicarbonate (Cryer & Mahaffey 2014). The bicarbonate acts to decrease the acid in the gastric lumen (Rahim et al. 2014). In this regard, it has been previously reported using cyclooxygenase-2 as a marker for inflammatory processes on the surface epithelium and lamina propria of the gastric mucosa (Talaat et al. 2014). Our results showed a significant positive immunoreactivity of cyclooxygenase-2 in most epithelial cells lining of the gastric mucosa in the ulcer model rats group. This result agrees with Mahmoud and Abd El-Ghffar (2019) who reported that NSAIDs caused prostaglandin inhibition in the gastric mucosa of mice.

Our immunohistochemical results showed a significant intensive immunoreactivity to PCNA distributed among all gastric glandular cells in the ulcer model group rats compared with the other groups (Antodine, pomegranate, and control). This result indicated proliferations in the gastric tissue following Brexin application (Polo et al. 2012). A similar result has been reported by Alazzouni et al. (2020), who reported an intensive immunoreactivity to PCNA in rat stomach tissue following Brexin application Pantolo as NSAIDs drug.

Several studies have indicated that the H2 receptor antagonist's efficacy depends on their ability to prevent gastric acid production (Goldstein et al. 2006a). Therefore, inhibiting acid secretion by H2 receptor antagonist is considered an effective defense against the ulcerative effect of the NSAIDs on gastric mucosa (Suzuki & Hibi 2005).

In the current study, the histochemical results of Antoine treated rats showed improved mucus production covering the surface mucosal lining, which explains the productive role of H2 receptor antagonist against gastric acid-induced by Brexin. Similar results were reported by KOTOB et al. (2018), who demonstrated that omeprazole, following NSAIDs drug application, showed an increase in the mucus carbohydrates distribution at the gastric tissue surface.
Our immunohistochemical results of Antoine treated rats showed mild immunoreactivity to cytokeratin-20 and cyclooxygenase-2 in the gastric tissue following Brexin application. The immunohistochemical results of the PCNA indicated proliferations occurring in the gastric tissue following Brexin application. This result agrees with Wang et al. (2015). They demonstrated that Pantoprazole showed an increase in immunoreactivity to PCNA whether following prostaglandin application or at treatment with H2 receptor antagonist alone in the gastric tissue.

Reports of Haque et al. (2015) showed that pomegranate fruit contains many bioactive principles, mainly flavonoids, alkaloids, tannins, triterpenes, and phytosterols having potential cytoprotective, anti-inflammatory, analgesic, and antioxidant properties Salgado et al. (2012). Phenolics and flavonoids are considered a critical defense against free radicals and protection against lipid peroxidation induced by NSAIDs in gastric tissue (Sumbul et al. 2011).

Our histopathological results showed a noticeable improvement in the glandular tissue erosion caused by Brexin in the pomegranate peel extract treatment group. A similar result was reported by Colombo et al. (2013), who showed that pomegranate peel hydroalcoholic extracts significantly decreased mucosal injury on the 6th day of treatment. Our histochemical study also showed an increase in the distribution of polysaccharides secretion among glandular tissue in the pomegranate peel extract treated group. Chauhan et al. (2016) showed that peel extracts of Punica granatum have gastric cytoprotective effects by enhancing the defensive mucin secretion, glycoproteins and decreasing the oxidative stress mainly by promoting antioxidant status.

Our electron microscopic results showed that the pomegranate peel extract administration, following Brexin application, improved the ultrastructural change in the mucous cell organelles, including nucleus membrane irregularities, mitochondria degeneration, and vacuolated zymogenic secretory granules. Also, electron microscopic results showed improvements in the surface epithelial lining and the gastric pits of the gastric tissue. Furthermore, the histochemical findings supported the electron microscopic results and showed the pomegranate peel extract administration, following Brexin application, improved production and distribution of the carbohydrates between the glandular.

The immunohistochemical results for the pomegranate group rats showed a moderate expression of cytokeratin-20, cyclooxygenase-2, and PCNA in the epithelial lining gastric mucosa. These results were explained by the fact that cytokeratin-20 expression is upregulated in the case of inflammation and repair processes (Komori et al. 2005) A lower level of cyclooxygenase-2 expression is fed back to gastric epithelium healing, according to Talaat et al. (2014). An elevation of PCNA expression assembles better healing and repairing processes, according to Polo et al. (2012). They claimed that increased PCNA expression accompanied by increased cellular proliferation is a sign of ulcer re-epithelization. Al-Hussaini (2014) has reported that pomegranate peel extract contains ellagic acid, ellagitannins, and gallic acids.

The presence of those polyphenols in pomegranate peel may be responsible for its antiulcer effects. Our results showed the presence of various polyphenolics compounds extracted using ethanol from pomegranate peels. Altogether, these results suggested that polyphenols in pomegranate peel could
participate in enhancing the mucosal barrier. In addition to the inhibition of the H2 receptor antagonist, the polyphenols exhibit free-radical-scavenging properties, a stimulatory effect on prostaglandin and, therefore of mucus secretion.

**Conclusion**

The uncontrolled application of anti-inflammatory drugs, including Brexin, is associated with gastric ulcer development. Here, we examined PPE's potential protective efficacy against peptic ulcers induced by Brexin drug by examining the histopathological, histochemical, immunohistochemical, and ultrastructural changes in rats' stomach tissues. The obtained findings revealed that PPE administration abolishes the peptic ulcer damages induced by Brexin drug. PPE's protective efficiency may correlate with its strong antioxidant properties, suggesting that PPE may be used to improve the peptic ulcer caused by the treatment with Brexin.

**Declarations**

**Acknowledgment**

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**Authors’ contributions**

Aya S. Fathalla and Tahani Al-Hazani: animal treatments, biochemical, methodology and software; Mohamed Abdel Daim, Mohamed S. Gabri: visualization, investigation, and histological examinations; Ashraf Albrakati: writing- reviewing and editing; Ahmed S. Alazzouni and Basma N. Hassan: conceptualization, validation, and supervision. All authors participated in the design, interpretation of the studies, and analysis of the data and review of the manuscript.

**Ethical approval**

All experimental protocols were undertaken in line with the Committee of Research Ethics for Laboratory Animal Care, Department of Zoology and Entomology, Faculty of Science, Helwan University (approval no., HU2020/Z/11), in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, 8th edition (NIH Publication no. 85–23, revised 1985).

**Consent to Participate**

Not applicable

**Consent to Publish**

Consented
Competing Interests

No conflict of interest to declare

Availability of data and materials

Available upon request

Funding

Not applicable

References


Figures
Figure 1

High performance liquid chromatography analysis of pomegranate peel extract.
Figure 2

Photomicrographs showing the histopathological examination of the gastric mucosal layer in stomach tissue of all the groups. Control group (A), ulcer model group (B), Antodine treated group (C) and pomegranate treated group (D), A and D (x400) and B and C (x600).
Figure 3

Transmission electron micrograph: A. shows chief cell of the gastric mucosa of control rat group. B. shows surface mucous cells of the gastric mucosa of ulcer-model group. C. shows gastric mucosa of the Antodine treated rat group. D. shows chief cell of the gastric mucosa of pomegranate treated rat group.
Figure 4

Scanning electron micrograph: A. shows surface epithelial cell lining of the gastric pits of control rat group. B. shows surface of the gastric of ulcer-model rat group. C. shows surface epithelial cell lining of the gastric pits of the Antodine treated rat group. D. shows surface of the gastric mucosa of pomegranate treated rat group.
Figure 5

Photomicrographs showing histochemical examination of the gastric mucosal layer in all different groups (PAS stain). Control group, ulcer model group, Antodine treated group, and pomegranate treated group, A, B, C, D, respectively. (x400).
Figure 6

Photomicrographs showing immunohistochemical examination of cytokeratin-20 of the gastric mucosal layer in all different groups. Control group, ulcer model group, Antodine treated group, and pomegranate treated group, A, B, C, D, respectively. (x400).
Figure 7

Photomicrographs showing immunohistochemical examination of cyclooxygenase-2 of the gastric mucosal layer in all different groups. Control group, ulcer model group, Antodine treated group, and pomegranate treated group, A, B, C, D, respectively. (x400).
Figure 8

Photomicrographs showing immunohistochemical examination of PCNA of the gastric mucosal layer in all different groups. Control group, ulcer model group, Antodine treated group, and pomegranate treated group, A, B, C, D, respectively. (x400).
Figure 9

Effects of PPE (43 mg/kg) on PAS, cytokeratin-20, cyclooxygenase-2 and PNCA in all groups, control group, ulcer model group, Antodine treated group, and pomegranate treated group, A, B, C, D, respectively. Values taking the same letter considered non-significantly different from each other.