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

Ahmed S. Alazzouni  
Helwan University Faculty of Science

Mohamed Abdel Daim  
King Saud University College of Science

Mohamed S. Gabri  
Helwan University Faculty of Science

Aya S. Fathalla  
Helwan University Faculty of Science

Ashraf Albrakati (✉ mchaki@hotmail.com)  
Taif University College of Medicine  https://orcid.org/0000-0002-4116-7865

Tahani Al-Hazani  
Prince Sattam bin Abdulaziz University

Basma N. Hassan  
Helwan University Faculty of Science

Research Article

Keywords: pomegranate, peptic-ulcer, stomach, rat, histopathology

Posted Date: May 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-474368/v1

License: ☺️ ☀️ This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
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 Brexit 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 peel extracts 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 famtodidine) 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.

Brexit group: 10 rats were administered a single oral dose of Brexit 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 &Elswawy
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
dischected, 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

This work was supported by Taif University Researchers Supporting Program (Project number: TURSP-2020/151), Taif University, Saudi Arabia.

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

1. Abbasi MM, Heidari R, Milani PZ, Shishavan NG (2015) EFFECTS OF POMEGRANATE SEED METHANOLIC EXTRACT ON METHOTREXATE-INDUCED CHANGES IN RAT LIVER ANTIOXIDANT COMPOUNDS. Current Topics in Nutraceutical Research 13

2. Abid M, Yaich H, Cheikhrouhou S, Khemakhem I, Bouaziz M, Attia H, Ayadi M (2017) Antioxidant properties and phenolic profile characterization by LC–MS/MS of selected Tunisian pomegranate peels. J Food Sci Technol 54:2890–2901

3. Adebajo A, Iwalewa E, Obuotor E, Ibikunle G, Omisore N, Adewunmi C, Obaparusi O, Klaes M, Adetogun G, Schmidt T (2009) Pharmacological properties of the extract and some isolated compounds of Clausena lansium stem bark: anti-trichomonal, antidiabetic, anti-inflammatory, hepatoprotective and antioxidant effects. J Ethnopharmacol 122:10–19

4. Adhikary B, Yadav SK, Roy K, Bandyopadhyay SK, Chattopadhyay S (2011) Black Tea and Theaflavins Assist Healing of Indomethacin-Induced Gastric Ulceration in Mice by Antioxidative Action. Evidence-Based Complementary and Alternative Medicine 2011, 546560

5. Ahmad N, Tahir M, Lone KP (2016) Amelioration of acetaminophen induced hepatotoxicity by methanolic extract of pomegranate peels in rats. J Pak Med Assoc 66:859–863

6. Al-Hussaini J (2014) Protective effect of Punica granatum peel extract against gastric mucosal erosions induced by ethanol in experimental rabbit models. AL-Qadisiyah Journal of Veterinary Medicine Sciences 13:52–58

7. Al-Omar MA, Al-Mohizea AM (2009) Famotidine, Profiles of drug substances, excipients and related methodology. Elsevier, pp 115–151

8. Alazzouni AS, Fathalla AS, Gabri MS, Dkhil MA, Hassan BN (2020) Role of bone marrow derived-mesenchymal stem cells against gastric ulceration: Histological, immunohistochemical and ultrastructural study. Saudi Journal of Biological Sciences 27:3456–3464

9. Albrakati A (2020) Neuroprotective effect of physical exercise on neuronal apoptosis induced by tramadol in cerebral cortex of rats. Biointerface Res Appl Chem 10:7209–7222
10. Alkushi AGR, Elsawy NAM (2017) Quercetin attenuates, indomethacin-induced acute gastric ulcer in rats. Folia Morphol 76:252–261
11. Avila J, De La Lastra CA, Martin M, Motilva V, Luque I, Delgado D, Esteban J, Herreras J (1996a) Role of endogenous sulphydryls and neutrophil infiltration in the pathogenesis of gastric mucosal injury induced by piroxicam in rats. Inflamm Res 45:83–88
12. Avila JR, de la Lastra CA, Martin MJ, Motilva V, Luque I, Delgado D, Esteban J, Herreras J (1996b) Role of endogenous sulphydryls and neutrophil infiltration in the pathogenesis of gastric mucosal injury induced by piroxicam in rats. Inflammation research: official journal of the European Histamine Research Society … et al.] 45, 83 – 8
13. Bancroft JD, Gamble M (2008) Theory and practice of histological techniques. Elsevier health sciences
14. Beck PL, Xavier R, Lu N, Nanda NN, Dinauer M, Podolsky DK, Seed B (2000) Mechanisms of NSAID-induced gastrointestinal injury defined using mutant mice. Gastroenterology 119:699–705
15. Brooks F (1985) The pathophysiology of peptic ulcer disease. Dig Dis Sci 30:15S–29S
16. Cardiff RD, Miller CH, Munn RJ (2014) Manual immunohistochemistry staining of mouse tissues using the avidin–biotin complex (ABC) technique. Cold Spring Harbor Protocols 2014, pdb. prot073429
17. Chauhan I, Sharma A, Gangwar M, Gautam MK, Singh A, Goel R (2016) Gastric antiulcer and ulcer healing effects of Punica granatum L. Peel extract in rats: Role of offensive and defensive mucosal factors and oxidative stress. Int J Pharm Pharm Sci
18. Colombo E, Sangiovanni E, Dell’Agli M (2013) A review on the anti-inflammatory activity of pomegranate in the gastrointestinal tract. Evidence-Based Complementary and Alternative Medicine 2013
19. Cryer B, Mahaffey KW (2014) Gastrointestinal ulcers, role of aspirin, and clinical outcomes: pathobiology, diagnosis, and treatment. Journal of multidisciplinary healthcare 7:137
20. DaSilva NA, Nahar PP, Ma H, Eid A, Wei Z, Meschultz S, Zawia NH, Slitt AL, Seeram NP (2019) Pomegranate ellagitannin-gut microbial-derived metabolites, urolithins, inhibit neuroinflammation in vitro. Nutr Neurosci 22:185–195
21. Drini M (2017) Peptic ulcer disease and non-steroidal anti-inflammatory drugs. Australian prescriber 40:91
22. El-Hamamsy S, El-khamissi H (2020) Phytochemicals, Antioxidant Activity and Identification of Phenolic Compounds by HPLC of Pomegranate (Punica granatum L.) Peel Extracts. Journal of Agricultural Chemistry Biotechnology 11:79–84
23. Goldstein J, Miner P Jr, Schlesinger P, Liu S, Silberg D (2006a) Intragastric acid control in non-steroidal anti-inflammatory drug users: comparison of esomeprazole, lansoprazole and pantoprazole. Aliment Pharmacol Ther 23:1189–1196
24. Goldstein JL, Miner PB Jr, Schlesinger PK, Liu S, Silberg DG (2006b) Intragastric acid control in non-steroidal anti-inflammatory drug users: comparison of esomeprazole, lansoprazole and
pantoprazole. Aliment Pharmacol Ther 23:1189–1196
25. Goldstein JL, Cryer B (2015) Gastrointestinal injury associated with NSAID use: a case study and review of risk factors and preventative strategies. Drug Healthc Patient Saf 7:31
26. Halter F, Tarnawski A, Schmassmann A, Peskar B (2001) Cyclooxygenase 2—implications on maintenance of gastric mucosal integrity and ulcer healing: controversial issues and perspectives. Gut 49:443–453
27. Haque N, Sofi G, Ali W, Rashid M, Itrat M (2015) A comprehensive review of phytochemical and pharmacological profile of Anar (Punica granatum Linn): A heaven's fruit. Journal of Ayurvedic Herbal Medicine 1:22–26
28. Hernández-Díaz S, Rodríguez LAGa (2002) Incidence of serious upper gastrointestinal bleeding/perforation in the general population:: Review of epidemiologic studies. J Clin Epidemiol 55:157–163
29. Hou C, Zhang W, Li J, Du L, Lv O, Zhao S, Li J (2019) Beneficial effects of pomegranate on lipid metabolism in metabolic disorders. Molecular nutrition food research 63:1800773
30. Ichikawa T, Hotta K, Ishihara K (2009) Second-generation histamine H (2)-receptor antagonists with gastric mucosal defensive properties. Mini Rev Med Chem 9:581
31. Jain KS, Shah AK, Bariwal J, Shelke SM, Kale AP, Jagtap JR, Bhosale AV (2007) Recent advances in proton pump inhibitors and management of acid-peptic disorders. Bioorg Med Chem 15:1181–1205
32. Jensen K (2008) Theory and practice of histological techniques. American Association of Neuropathologists, Inc
33. Kandeil MA, Hassanin KM, Arafa MM, Abdulgawad HA, Safwat GM (2019) Pomegranate peels ameliorate renal nitric oxide synthase, interleukin-1β, and kidney injury molecule-1 in nephrotoxicity induced by acrylamide in rats. Egyptian Pharmaceutical Journal 18:368
34. Kobayashi T, Tonai S, Ishihara Y, Koga R, Okabe S, Watanabe T (2000) Abnormal functional and morphological regulation of the gastric mucosa in histamine H2 receptor–deficient mice. J Clin Investig 105:1741–1749
35. Komori M, Tsuji S, Tsujii M, Murata H, Iijima H, Yasumaru M, Nishida T, Irie T, Kawano S, Hori M (2005) Efficiency of bone marrow-derived cells in regeneration of the stomach after induction of ethanol-induced ulcers in rats. Journal of gastroenterology 40:591–599
36. KOTOB SE, SAYED A, MOHAMED S, AHMED H (2018) Quercetin and ellagic acid in gastric ulcer prevention: An integrated scheme of the potential mechanisms of action from in vivo study. Asian Journal of Pharmaceutical Clinical Research 11:381
37. Mahmoud YI, Abd El-Ghffar EA (2019) Spirulina ameliorates aspirin-induced gastric ulcer in albino mice by alleviating oxidative stress and inflammation. Biomed Pharmacother 109:314–321
38. Matyushchenko N, Stepanova T (2003) Quantitative determination of the total content of flavonoids in the new phytopreparation Elima. Pharm Chem J 37:261–263
39. Milosevic V, Vukmirovic F, Zindovic M, Krstic M, Milenkovic S, Jancic S (2015) Interplay between expression of leptin receptors and mucin histochemical aberrations in colorectal adenocarcinoma. Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie 56, 709 – 16

40. Moote C (1992) Efficacy of nonsteroidal anti-inflammatory drugs in the management of postoperative pain. Drugs 44:14–30

41. Musumba C, Pritchard D, Pirmohamed M (2009) cellular and molecular mechanisms of NSAID-induced peptic ulcers. Aliment Pharmacol Ther 30:517–531

42. Nagano Y, Matsui H, Muramatsu M, Shimokawa O, Shibahara T, Yanaka A, Nakahara A, Matsuzaki Y, Tanaka N, Nakamura Y (2005) Rebamipide significantly inhibits indomethacin-induced mitochondrial damage, lipid peroxidation, and apoptosis in gastric epithelial RGM-1 cells. Dig Dis Sci 50(Suppl 1):S76–S83

43. Nash J, Lambert L, Deakin M (1994) Histamine H 2-Receptor Antagonists in Peptic Ulcer Disease. Drugs 47:862–871

44. Polo C, Moraes T, Pellizzon C, Marques M, Rocha L, Hiruma-Lima CA (2012) Gastric ulcers in middle-aged rats: The healing effect of essential oil from Citrus aurantium L.(Rutaceae). Evidence-Based Complementary and Alternative Medicine 2012

45. Qabaha K, Al-Rimawi F, Nusseibeh S, Abbadi J, Abu-Lafi S (2019) Phenolic and flavonoids analysis of pomegranate peel extracts and their antinflammatory and antioxidant activities. International Journal of Pharmaceutical Quality Assurance 10:60–65

46. Rahim NA, Hassandarvish P, Golbabapour S, Ismail S, Tayyab S, Abdulla MA (2014) Gastroprotective effect of ethanolic extract of Curcuma xanthorrhiza leaf against ethanol-induced gastric mucosal lesions in Sprague-Dawley rats. BioMed Research International 2014

47. Russell R (2001) Non-steroidal anti-inflammatory drugs and gastrointestinal damage—problems and solutions. Postgraduate medical journal 77:82–88

48. Sabiu S, Garuba T, Sunmonu T, Ajani E, Sulyman A, Nurain I, Balogun A (2015) Indomethacin-induced gastric ulceration in rats: protective roles of Spondias mombin and Ficus exasperata. Toxicology reports 2:261–267

49. Saeed AA, Abdu OH, Salem TABF (2020) HPLC Analysis and DPPH Assay of Some Bioactive Compounds in Pomegranate Peel Extracts. Research and Reviews: Journal of Medicinal Chemistry 2

50. Salgado JM, Ferreira TRB, de Oliveira Biazotto F, dos Santos Dias CT (2012) Increased antioxidant content in juice enriched with dried extract of pomegranate (Punica granatum) peel. Plant foods for human nutrition 67, 39–43

51. Salvatella M, Rossi I, Del Valle JC, Gutiérrez Y, Pereda C, Samper B, Feliu JE (2004) Inhibition of acid secretion by the nonsteroidal anti-inflammatory drugs diclofenac and piroxicam in isolated gastric glands: analysis of a multifocal mechanism. American Journal of Physiology-Gastrointestinal Liver Physiology 286:G711–G721
52. Schoen RT, Vender RJ (1989) Mechanisms of nonsteroidal anti-inflammatory drug-induced gastric damage. The American journal of medicine 86, 449 – 58
53. Schunack W (1989) Pharmacology of H2-receptor antagonists: an overview. The Journal of international medical research 17, 9A-16A
54. Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology Viticulture 16:144–158
55. Sumbul S, Ahmad MA, Mohd A, Mohd A (2011) Role of phenolic compounds in peptic ulcer: An overview. J Pharm Bioallied Sci 3:361–367
56. Suzuki H, Hibi T (2005) Novel effects other than antisecretory action and off-label use of proton pump inhibitors. Expert opinion on pharmacotherapy 6:59–67
57. Talaat R, Abdel-Hakem N, El-Toumy S, Samaka R, Mohamed A, El-Shahat M (2014) Anti-angiogenic and Anti-Inflammatory Activity of Punica granatum Peel on Experimentally -Induced Gastric Ulcer in Rats. International Journal of Biological Chemical Sciences 5:42–56
58. Todorovic V, Sokic-Milutinovic A, Drndarevic N, Micev M, Mitrovic O, Nikolic I, Wex T, Milosavljevic T, Malfertheiner P (2006) Expression of cytokeratins in Helicobacter pylori–associated chronic gastritis of adult patients infected with cagA + strains: An immunohistochemical study. World Journal of Gastroenterology: WJG 12:1865
59. Viuda-Martos M, Fernández-López J, Pérez-Álvarez J (2010) Pomegranate and its many functional components as related to human health: a review. Comprehensive Reviews in Food Science Food Safety 9:635–654
60. Wang D, Özen C, Abu-Reidah IM, Chigurupati S, Patra JK, Horbanczuk JO, Jóźwik A, Tzvetkov NT, Uhrin P, Atanasov AG (2018a) Vasculoprotective Effects of Pomegranate (Punica granatum L.). Front Pharmacol 9:544–544
61. Wang S-Y, Wang H-Y, Wang T-E, Wang H-H, Chang W-H, Chu C-H, Lin S-C, Yeh H-I, Shih S-C (2015) Delayed healing of gastric ulcer is associated with downregulation of connexin 32 in the gastric mucosa. Advances in Digestive Medicine 2:67–73
62. Wang T, Men R, Hu M, Fan X, Yang X, Huang X, Ye T, Yang L (2018b) Protective effects of Punica granatum (pomegranate) peel extract on concanavalin A-induced autoimmune hepatitis in mice. Biomedicine pharmacotherapy = Biomedecine pharmacotherapie 100:213–220

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.