Scrutinizing the antidiabetic, antidiarrheal, and anti-inflammatory activities of methanolic extract of pomegranate peel via different approaches

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Abstract

The objective of the current study was to evaluate the potential of *Punica granatum* L peel in mice as an antidiarrheal and antidiabetic agent. In an antidiarrheal study, different doses (50, 100, 150, and 200 mg/kg) of methanolic pomegranate peel extract (PPE) were administrated to castor oil-induced (1 mL/kg) diarrheal mice. Mice administered loperamide hydrochloride (3 mg/kg) were treated as a baseline group. During the experiment, electrolyte and hematological levels were analyzed, and at the end, histopathology of the intestine was performed. For antidiabetic activity, PPE doses (50, 100, 150, and 200 mg/kg) and metformin hydrochloride were administered to alloxan-induced (150 mg/kg) diabetic mice groups, and biochemical and hematological parameters were analyzed. Liver histopathology was done at the end of the experiment. The study found that castor oil caused diarrhea and had a significant (*p* < 0.05) impact on hematological parameters and electrolyte levels, compared with negative control group. PPE helped to restore altered parameters to normal levels. Histopathology of positive control group revealed abnormal cell structures, with irregularly arranged villi, unclear mucosal architecture of the ileal section, and nuclei cells were damaged and prone to collapsing. Significant dose-dependent recovery was observed in PPE-fed mice groups. After inducing and confirmation of diabetes with alloxan, all groups, except the negative control group, had significantly high glucose levels (*p* < 0.05). Levels of C-reactive protein and bilirubin were significantly altered, but PPE and metformin hydrochloride showed potential to improve these parameters. In positive control group mice, liver histology showed microvesicular fatty changes throughout the acinus, reactive Kupffer cells, mid-portal inflammation, reduced portal triad, centrilobular visibility, and well-differentiated central vein with well-formed nuclei. Similarly, significant dose-dependent recovery was observed...
in PPE-administrated mice groups. These results demonstrated that PPE had promising antidiarrheal and antidiabetic potential.

Keywords: alloxan; castor oil; antidiabetic; antidiarrheal; pomegranate peel extract

Introduction

Medicinal plants are a valuable source of medications (Semwal et al., 2010) because they are a reservoir of biologically active compounds with therapeutic uses (Ahmad et al., 2023a, 2023b; Hayat et al., 2023; Muhammad et al., 2023; Naveed et al., 2022a). Medicinal plants have been used extensively against various infections and for traditional therapies across the world. These medicinal plants are being used for centuries and could potentially be considered the genesis of modern medicine (Ammara et al., 2023; Aziz et al., 2023; Ejaz et al., 2023; Naveed et al., 2022b; Saleem et al., 2022; Salmerón-Manzano et al., 2020; Zawar et al., 2023).

Based on the World Health Organization (WHO) reports, 70% of the world’s population uses 35–70 plant species for their health care (Iram et al., 2023; Mamedov, 2012; Riasat et al., 2023). As opposed to manufactured pharmaceuticals, researchers have concentrated more on medicinal plants, as they don’t have adverse reactions (Arnold, 2013). Additionally, 25% of the ingredients used widely in pharmaceuticals are derived from medicinal plants (Robbers et al., 1996). Many phytochemicals are reported to be present in profusion in these plants (Munuswamy et al., 2013). Phytochemicals are non-nutritive compounds thought to be a type of defence system of plants used against hazards, such as illness, herbivorous animals, and ultraviolet (UV) radiation (Elfalleh et al., 2012).

In recent years, researchers have increasingly prioritized medicinal plants because of their eco-friendliness, compared to synthetic pharmaceuticals, often having dangerous adverse reactions (Arnold, 2013). From this perspective, the importance of therapeutic herbs is unavoidable. Pomegranate, scientifically known as *Punica granatum* L. and belonging to the Lythraceae family, is used globally due to its rich medicinal and nutritional heritage (Boroughs et al., 2016, Ismail et al., 2012). It is the healthiest fruit with high phenolic compounds (Kaderides et al., 2021). The constituents of pomegranate provide a wide range of therapeutic benefits (Lansky and Newman, 2007). Naturally, pomegranate is grown in many countries, including Iran, Afghanistan, Morocco, India, Italy, China, and Pakistan (Shayganna et al., 2016). Parts of pomegranate, including the bark, seed, and leaves, are used to treat various diseases. Its peel is used to cure different diseases, including syphilis and bronchitis, as the peel is thought as one of the finest sources of bioactive compounds (Sreekumar et al., 2014). Furthermore, pomegranate peel is renowned for its anticancer, antiatherosclerotic, wound-healing, and antioxidant properties (Cerdá et al., 2003, Navarro et al., 1996, Rajan et al., 2011). Antioxidant compounds found in peel are flavonoids, proanthocyanidins, ellagitannins, and phenols (Mekawi et al., 2019).

Pomegranate peel constitutes nearly half of the entire weight of the fruit which has been used to treat diabetes, diarrhea, and other diseases (Boroughs et al., 2016, Hou et al., 2019). Diabetes is a category of metabolic disease induced by high glucose levels in the blood, characterized by damaged beta cells, causing a decrease in insulin production (Udeogu et al., 2019). It is a serious health issue because of its high prevalence (Tan et al., 2019), with 425 million diabetics worldwide in 2017, which will be 642 million by 2040 (Magliano and Boyko, 2022). Synthetic antidiabetic medications used for diabetes are expensive and cause adverse reactions, including hypoglycemic coma and kidney and liver diseases (Rasouli et al., 2020). An important area of study involves the pursuit of safer and more effective medication (Gaonkar and Hullatti, 2020). Diarrhea is abnormally loose or watery feces caused by bacteria, viruses, or parasites. Although various antidiarrheal medications are available, the disease remains at the top of causing mortality and morbidity worldwide (Bustreo et al., 2015). Consequently, this study aims to assess the in vivo effects of the methanolic extract of pomegranate peel extract (PPE) on alloxan-induced diabetes in mice to determine its potential antidiabetic activity. Similarly, the experiment evaluated the PPE impact on castor oil-induced diarrhea in mice to explore its potential as an antidiarrheal agent. Also, this study aimed to identify the effects of PPE on the structure and function of the liver and intestine.

Material and Methods

Plant materials

Pomegranate peel sample was collected from the local area (Mingora) of District Swat, Khyber Pakhtunkhwa (KPK), Pakistan. This peel sample was kept at the herbarium of the Department of Botany, University of
Malakand, Chakdara, Pakistan until further processing. Peel was cleaned properly twice with sterile water and for one time with tap water, and then kept for drying for 4–6 days in a covered place at room temperature (25°C).

**Extract preparation**

The dry peel was crushed into powder form using an electric grinder. After that, methanolic extract was prepared using the procedure described by Mutahar _et al._ (2012) with slight adjustments; the methanol concentration was increased to 95%, sonication was performed for 10 min, and the centrifugation was increased to 15 min. The resulting peel powder was mixed with methanol at a ratio of 1:10. The mixture was placed in a 250-mL flask and agitated using a shaker for 24 h at room temperature at 15 revolutions per minute (rpm). After being shaken for 24 h, the mixture was sonicated for 10 min at room temperature. Following that, the mixture was filtered by using Whatman No. 1 filter paper. After filtration, the extract was centrifuged at 1,300 rpm for 5 min to separate supernatant. The supernatant was shaded for 72 h on a petri plate. The concentrated peel crude extract was obtained at evaporation's end and kept at 40°C for further usage.

**Animals and experimental design**

Both male and female albino mice were used in the current study. A day-old albino mice were raised at the University of Malakand BioPark for this experiment. The mice were reared in an appropriate environment for 40 days, in a proper temperature of 22–25°C and 12-h light-dark cycles, and provided a proper diet. On the 40th day of upbringing, different groups of animals were formed, each with five animals having the same weight. In the current study, two activities were performed.

**Chemicals and drugs**

The following chemicals and drugs were used in the study: alloxan (Sigma Chem. Co., St. Louis, MO, USA), loperamide hydrochloride (Imodium) purchased from a pharmacy, metformin hydrochloride (Getz Pharma, Pakistan), and castor oil (Micko Industrial Chemicals) purchased from a local pharmacy.

**Antidiabetic activity**

This study assessed the antidiabetic activity of PPE. G1 was the negative control group; normal water and a typical diet were provided to G1 mice. The second group (G2) was the positive control group; alloxan was given intravenously at a dosage of 150-mg/kg body weight. The mice were given normal food and water. Alloxan was given intraperitoneally to the remaining five mice groups (G3–G7) at a dosage of 150 mg/kg to induce diabetes. After 48 h of intoxication, diabetes was confirmed in these mice groups. These mice groups were fed different amounts of PPE per kilogram of body weight regularly for 1 month: 50-mg PPE was given to G3, 100 mg to G4, 150 mg to G5, and 200 mg to G6. Metformin hydrochloride was given to G7 animals at a dosage of 50-mg/kg body weight. Throughout the experiment, animals received their usual water and food supplies. Animals were monitored under surveillance to check changes in their weight loss or gain, eye color, their locomotion, and feeding activity. Also, the blood glucose levels of these animals were checked while receiving PPE regularly.

**Analysis of biochemical and hematological parameters**

After feeding PPE for 1 month, blood samples were taken after the animals were euthanized in heparinized tubes; blood samples were analyzed for hematology parameters using a fully automated blood hematology analyzer (SYS MIX, Japan). Also, gel tubes were used to collect blood samples to separate serum for measuring biochemical parameters. Standard reagent kit methods were used to measure C-reactive protein (CRP), glucose levels, and bilirubin (direct and indirect) using the analyzer.

**Examining histopathology**

After euthanasia of animals, liver samples were taken out and stored in distilled water-diluted formalin solution (1:10). Tissue was sectioned using a microtome (Rotary Microtome Minux® S700A, China), and the slides were stained by the usual protocol (Slaoui and Fiette, 2011). The slides were analyzed at Anwar Clinical Laboratory (Saidu Sharif Swat, Khyber Pakhtunkhwa, Pakistan). DCM 130 (USB) microscopic digital camera, with a resolution of 1.3 MP, was used to capture images.

**Antidiarrheal activity**

The primary objective of this research was to establish the antidiarrheal efficacy of PPE. As mentioned, G1 mice were treated as a negative control, and fed with normal water and food supply. G2 mice were given castor oil (1-mL/100 g body weight), and these served as a positive control; animals received normal water and food supply. Mice of other groups (G3–G7) were given castor oil (1-mL/kg body weight) to induce diarrhea. A white paper was spread underneath the animals to confirm
diarrhea, which was induced 15 min after giving castor oil. Following initiation of diarrhea, different amount of PPE was given to each group: 50 mg to G3, 100 mg to G4, 150 mg to G5, and 200 mg to G6 mice. The trial was carried out consistently over a period of 7 days, with repeated iterations. Loperamide hydrochloride was administered at a dosage of 3 mg/kg to (G7 animals (Antonisamy et al., 2015). All animal groups received regular supplies of food and water.

Analysis of hematological and electrolyte parameters
In order to test serum electrolytes, blood samples (2 mL) were collected in gel tube vacutainers. Evaluated serum electrolytes were ionized Ca ion (ICa), Na+, Cl−, and K+. Blood samples underwent 15 min of centrifugation at 5,000 rpm to obtain clear serum samples. The isolated serum was examined using a CBS 400 auto electrolyte analyzer (B&E Scientific Inc., China).

Examining histopathology
Intestine samples were collected and stored in diluted (distilled water) formalin solution (1:10). Tissues were sectioned using microtome (Rotatory Microtome Minux® S700A, China). Slide preparation and staining were carried out according to a protocol (Slaoui and Fiette, 2011). Slides were examined at Anwar Clinical Laboratory (Swat, Khyber Pakhtunkhwa, Saidu Sharif, Pakistan), and images were taken with 1.3-MP resolution DCM 130 microscope digital camera (USB 2.0).

Statistical analysis
The mean and standard deviation of collected data were obtained. Mean values of different parameters were compared with ANOVA and Tukey’s test. A significant difference was shown by \( p \leq 0.05 \).

Results

Antidiarrheal activity
This study examined the impact of different doses of PPE (50, 100, 150, 200 mg/kg body weight) in castor oil-induced diarrhea in mice.

Electrolyte analysis
Electrolyte analysis was conducted by isolating blood serum from diarrhea-induced mice and treated with PPE. Animal groups that received varying amounts of PPE and loperamide hydrochloride did not demonstrate any significant alteration \( (p < 0.05) \) in sodium, potassium, and ionized calcium ions, compared to the control group. However, the chloride ion showed a significant variance, compared to the control group as illustrated in Figure 1.

Hematological values
Hematological parameters of mice with diarrhea are shown in Table 1. A significant difference \( (p < 0.05) \) was observed in the levels of white blood corpuscles (WBCs) in the PPE doses of 50 mg/kg and 150 mg/kg, while no significant difference was observed in the doses of 100 mg/kg, and 200 mg/kg as well as the loperamide hydrochloride-mediated group (G7), compared to the control group. Granulocytes (GRA) and hematocrit (HCT) mean values demonstrated no significant differences \( (p < 0.05) \) in different PPE doses- and loperamide hydrochloride-treated groups. On the other hand, a significant difference \( (p < 0.05) \) was observed in the levels of hemoglobin in all PPE-treated groups, compared to the control group. PPE doses of 50, 100, and 200 mg/kg demonstrated significant differences \( (p < 0.05) \) in the levels of lymphocytes (LYM), compared to the control group. In case of red blood cells (RBCs), PPE doses of 50 mg/kg and 100 mg/kg showed significant differences \( (p < 0.05) \), compared to the control group. A significant difference \( (p < 0.05) \) was observed between PPE dosage groups of 100, 150, and 200 mg/kg, compared to the control group. The mean value of platelets (PLT) in groups treated with PPE doses of 50, 100, and 150 mg/kg showed a significant difference \( (p < 0.05) \), compared to the control group.

Effects of pomegranate peel extract on histology of the intestine
Samples of the intestines isolated from each group were examined. Mice in the negative control group (G1; fed normally) showed no changes in the gut, mucosal epithelium, villi, and nuclei cell structure. However, significant gastrointestinal changes were observed in the positive control group G2 (fed with 1-mL/kg castor oil), arrangement of the villi was inconsistent and unclear, and mucosal epithelial cells exhibited reduced clarity. Cellular delineation lines and disrupted mucosal morphology of the ileal section were also observed. Nuclei cells were damaged and vulnerable to collapse. G3 with 50-mg/kg PPE dosage showed villus atrophy because of changes in mucosal architecture and cellular structure. However, nuclei were found to be normal. In G4, which received 100-mg/kg PPE, noticeable changes in cellular structure, including reduced villus atrophy, were observed. On the other hand, G5 (150-mg/kg PPE) showed fewer changes in cellular structure, compared to changes in G3 and G4. However, reduced visibility of the villi was observed in G5 due to cytoplasmic vacuoles. In other groups, the only noticeable change in G6 (PPE dosage of 200 mg/kg)
Studying medicinal potential of methanolic pomegranate peel extract

Figure 1. Effects of methanolic pomegranate peel extract on levels of different electrolytes in diarrhea-induced mice. (A) Sodium ion (Na⁺), (B) potassium ion (K⁺), (C) chlorine ion (Cl⁻), (D) ionized calcium ion (ICa). Values of the control group are compared with PPE-fed doses (50, 100, 150, and 200 mg/kg body weight) and loperamide hydrochloride (L-A) dose, and presented as mean ± standard error mean (SEM); n = 5 and p < 0.05.

Table 1. Effect of pomegranate peel extract on the hematological parameters of mice following castor oil feeding.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Varying doses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative control</td>
</tr>
<tr>
<td>WBC (×10⁹/L)</td>
<td>7.36±0.25a</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>37.17±2.46a</td>
</tr>
<tr>
<td>GRA (%)</td>
<td>44.43±5.37a</td>
</tr>
<tr>
<td>RBC (×10¹²/L)</td>
<td>4.10±0.36a</td>
</tr>
<tr>
<td>Hgb (g/dL)</td>
<td>14.23±0.87a</td>
</tr>
</tbody>
</table>

Values of different parameters in all mice groups, including the loperamide hydrochloride-administrated group, and PPE-treated groups with various doses (50, 100, 150, and 200 mg/kg body weight), compared to the control group are presented as mean ± standard error mean (SEM); n = 5, p < 0.05 is represented by different superscripted letters (a,b,c).

was the presence of vacuoles in mucosal epithelial cells. In G7 (treated with 3-mg/kg loperamide hydrochloride), no changes were observed in cellular structure, and the villi exhibited a well-defined shape and structure. All the results are shown in Figure 2.

Antidiabetic activity of PPE

In this study, all mice groups, except the negative control group, were treated with a single dose of alloxan (150 mg/kg) to induce diabetes. One of the groups also received
Figure 2. Photomicrographs of the intestine in diarrhea-induced mice groups fed with PPE doses and the loperamide hydrochloride group. (A) The intestine in the negative control group (G1) showed no changes in the gut, mucosal epithelium, villi, and nuclei cells; (B) the intestine in the positive control group (G2) showed an inconsistent arrangement of the villi and mucosal epithelial cells were unclear and disrupted. The nuclei cells were damaged and prone to collapse in the ileal section; (C) the intestine in G3 (fed with 50-mg/kg PPE) showed changes in mucosal lining caused by villus atrophy, but nuclei were normal; (D) the intestine in G4 (fed with 100-mg/kg PPE) showed changes in cellular structure with reduced villus atrophy; (E) in G5 (fed with 150-mg/kg PPE), fewer changes were observed in cellular structure and cytoplasmic vacuoles, with less visibility of villi in the intestine; (F) intestine histology in G6 (fed with 200-mg/kg PPE) showed typical cell structure with clear and regular villi and vacuoles present in mucosal epithelial cells with no changes; (G) in G7, the intestine of the loperamide hydrochloride-treated mice showed no changes in the cellular structure, and shape and structure of the villi were also normal.
metformin hydrochloride. Blood glucose levels were assessed in all groups of mice on day 2, 8, 15, 22, and 28. After 48 h of feeding PPE to the mice, a significant difference ($p < 0.05$) was observed between the control group and other PPE-treated groups. Furthermore, significant decrease in glucose levels was observed in the animals on a daily basis in a dose-dependent manner as depicted in Figure 3. A PPE dose of 200 mg/kg was found to be the most effective in normalizing glucose levels.

**Biochemical parameters**

Based on the statistical analysis, no significant difference was observed ($p < 0.05$) between the average levels of biochemical parameters (total bilirubin, and direct and indirect bilirubin) in mice fed with PPE and those treated with metformin hydrochloride, compared to the negative control group. However, a significant increase ($p < 0.05$) was observed in CRP values, as illustrated in Figure 4.

**Hematological parameters**

During the 28-day study, significant differences were observed in the levels of RBCs, platelets, and hematocrit in various groups treated with PPE and metformin hydrochloride, compared to the control group, as shown in Table 2. However, differences in dosage were noted in other hematological parameters. For instance, a dose of 50 mg/kg of PPE resulted in significant differences in WBCs, while doses of 100, 150, and 200 mg/kg showed significant differences in GRA and mean corpuscular volume (MCV), compared to the control group. Lymphocytes exhibited significant differences in the PPE-fed mice groups, but not in the metformin hydrochloride group. Furthermore, no significant association ($p < 0.05$) was observed between the mean values of hemoglobin (Hgb) in the PPE-fed mice groups and the metformin hydrochloride-mediated mice group, compared to the control group.

**Histopathology of liver**

The analysis of liver histology showed mild to moderate toxic effects. The negative control group (G1) exhibited no changes in sinusoids, hepatic cords, and Kupffer cells. In the positive control group (G2, treated with 150-mg/kg alloxan), the histology of liver tissues revealed the existence of microvesicular fatty alterations throughout the acinus and central lobule, portal triad regions appeared less distinct/visible, and Kupffer cells exhibited sign of reactivity. In addition, mid-portal inflammation was observed and the central vein displayed well-differentiated features in histology of G2 animals. Nuclei structures were well formed and the hepatic lobules showed well-formed structures, with noticeable alterations in fatty microvesicular within the hepatic cords in histology of G3 (fed with 50-mg/kg PPE) animals. Also, G3 demonstrated regular orientation reactivity was observed in Kupffer cells.

**Figure 3.** Antidiabetic activity on different days (day 2, 8, 15, 22, and 28) after being fed with different doses of PPE (50, 100, 150, and 200 mg/kg) in alloxan-induced diabetic mice groups. Values are presented as standard error mean (SEM); $n = 5$, $p < 0.05$, compared to the standard control group.
Figure 4. Effects of methanolic pomegranate peel extract on biochemical parameters in diabetes-induced mice. Values of the control group are compared with PPE-fed groups (50, 100, 150, and 200 mg/kg), and metformin hydrochloride-administrated group, and presented as standard error mean (SEM); n = 5, \( p < 0.05 \).

Table 2. The effect of pomegranate peel extract on hematological parameters after mice were administered alloxan.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Different doses</th>
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<tbody>
<tr>
<td></td>
<td>Negative control</td>
</tr>
<tr>
<td>WBC (( \times 10^{9} /L ))</td>
<td>6.76\pm0.75a</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>28.30\pm2.96a</td>
</tr>
<tr>
<td>GRA (%)</td>
<td>40.23\pm5.14a</td>
</tr>
<tr>
<td>RBC (( \times 10^{12} /L ))</td>
<td>4.33\pm0.41a</td>
</tr>
<tr>
<td>Hgb (g/dL)</td>
<td>13.00\pm0.50a</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>40.67\pm1.25a</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>81.67\pm3.27a</td>
</tr>
<tr>
<td>PLT (( \times 10^{9} /L ))</td>
<td>323.02\pm27.51a</td>
</tr>
</tbody>
</table>

LYM: lymphocytes; Hgb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; PLT: platelets. Values of different parameters in all mice groups, including metformin hydrochloride, and PPE-administrated groups, compared to the control group, are presented as mean \pm standard error mean (SEM); n = 5, \( p < 0.05 \) is represented by different superscripted letters:\( ^{a,b,c} \).
and well-differentiated endothelial lining in the central vein with normal morphology. Similarly, in G4 (fed with 100-mg/kg PPE), liver histology revealed an arrangement of well-organized Kupffer cells and reactive changes. Well-organized hepatic cords demonstrated clear acinar microvesicular fatty changes, and proper endothelial lining was found in the central vein. The histology of G5 (fed with 150-mg/kg PPE) showed normal central vein and endothelial lining, with no fibrosis around blood vessels; a significant microvesicular fatty change from the central vein to portal triads and pan acinar microvesicular fatty changes were observed; reactive Kupffer cells and dilated sinusoid cells were also observed, with a healthy and normal central vein and well-formed hepatic cords. In G6 (fed with 200-mg/kg PPE), most hepatocytes showed microvesicular fatty alterations and normal nuclei; reactive Kupffer cells were observed in sinusoid walls that appeared relatively dilated with elevated bile pigment in their lumen. In G7 (fed with 50-mg/kg metformin hydrochloride), the central vein and endothelial lining were well formed, walls were not thickened, nuclei appeared normal, with reactive Kupffer cells and well-formed hepatic cords. The detailed data of all groups are shown in Figure 5.

**Discussion**

Medicinal plants are extensively used globally as an integral component of primary healthcare because of their natural origin, leading to fewer adverse effects, compared to synthetic drugs (Aqib et al., 2023; Hussain et al., 2023; Rauf et al., 2023; Waseem et al., 2023). The present study investigated the in vivo effects of methanolic PPE on mice to assess their potential in alleviating diabetes and diarrhoea. Diabetes was induced by intraperitoneal administration of 150-mg/kg body weight alloxan whereas diarrhoea was induced by oral administration of 1-mL/kg castor oil. To assess antidiabetic and antidiarrheal activities, mice were given various doses of PPE (50, 100, 150, and 200 mg/kg). The administrated castor oil resulted in loose, uniform stools of watery consistency, corresponding to the results of the study conducted by Zhao et al. (2018). According to the results of the studies conducted by Ammon et al. (1974), Pierce et al. (1971), and Tunaru et al. (2012), castor oil-induced diarrhoea was due to the presence of ricinoleic acid. The antidiarrheal effects of PPE in different mice groups were assessed by examining changes in hematology and levels of electrolyte parameters. No notable association (p < 0.05) was determined in the mean values of electrolytes (Na⁺, K⁺, and ICA), except for chloride (Cl⁻) ions. Contrary to a positive control, PPE was able to revive the electrolytes (Na⁺, K⁺, Cl⁻, and ICA) to normal levels. The study revealed a dose-dependent relationship between positive changes toward normalcy, with high effectiveness in the highest dose (200 mg/kg) of PPE, compared to other doses. Similar impact of PPE was discovered as that of loperamide hydrochloride (an effective antidiarrheal drug). It was indicated in prior studies (Baker, 2007, Pannemans and Corsetti, 2018, Sahi et al., 2020) that loperamide hydrochloride had the effect of decelerating colonic transit time. In addition, it lessened the digestive tract’s peristalsis and fluid output while improving the absorption of fluids and electrolytes. According to Qnais et al. (2007), PPE’s antidiarrheal activity could be due to numerous mechanisms, such as inhibiting the release of prostaglandins in the intestine, decrease in mucosal secretion, and promoting reabsorption of water and sodium chloride, thus facilitating the overall absorption of fluids and electrolytes. Furthermore, antidiarrheal effects of PPE were attributed to the presence of bioactive compounds (corilagin, punicalagin, and ellagic acid) by Zhao et al. (2018). In addition, Palombo’s (2006) findings revealed that the presence of high tannin and alkaloid contents in PPE could be responsible for antidiarrheal characteristics. In this study, a noticeable rise in Cl⁻ values was determined in positive control groups. These positive control groups correlated with the previous studies (Nagami, 2016; Walker et al., 1990). Phenolic compounds, such as flavonoids, tannins, and alkaloids, present in PPE could have positive effects to change levels of the electrolytes (Palombo, 2006).

In the present research, significant increase was observed in RBCs, hemoglobin, WBCs, platelets, and hematocrit. Conversely, a significant decrease was observed in total leucocyte count (TLC), lymphocytes, and mean corpuscular volume. The PPE dose of 200 mg/kg was more effective than other doses (50, 100, and 150 mg/kg), as it could significantly normalize the altered parameters. The groups that received the stated doses of PPE, except 200 mg/kg, showed distinct changes in their intestinal histology, such as changes in cell structure, visible villi, and mucosal architecture alteration, which were aligned with the previous findings of El-Kady et al. (2021) and Zhao et al. (2018). Results of the prior studies showed that urolithins were produced from pomegranate by metabolizing ellagitannins of intestinal microbiota in pigs, mice, and humans (Cerdà et al., 2003, 2005, Espín et al., 2007). In a study conducted by Yan et al. (2013), PPE demonstrated effectiveness in regulating intestinal function and supporting homeostasis. In contrast to the findings of Zhao et al. (2018), a normal cellular structure was observed in a group treated with 200-mg/kg PPE. Variations in this study could be attributed to differences in experimental models, duration PPE feeding regimen, and environmental conditions.

In the present study, diabetes was induced in mice groups by injecting alloxan intraperitoneally. According to a prior research conducted by Szabadi et al. (2014), Kliber et al. (1996), and Naseer et al. (2014), alloxan triggers a short-term release of insulin while inhibiting the islet’s
Figure 5. Photomicrographs of the liver in diabetes-induced mice groups fed with PPE and metformin hydrochloride. (A) Negative control mice group, no alteration was observed in hepatic cords, Kupffer cells, or sinusoids; (B) positive control mice group (G2, treated with alloxan) showed microvesicular fatty changes, reactive Kupffer cells, mid-portal inflammation, portal triad and central lobule less visibility, intact nuclei, and well-differentiated central vein; (C) G3 (fed with 50-mg/kg PPE) showed hepatic lobules with distinct structures with evident fatty microvascular changes in the hepatic cords, intact Kupffer cell orientation, and normally formed endothelial lining in the central vein; (D) G4 (fed with 100-mg/kg PPE) showed well-organized Kupffer cells, and reactive changes. Well-organized hepatic cords demonstrated clear acinar microvesicular fatty changes, and proper endothelial lining was discovered in the central vein; (E) G5 (fed with 150-mg/kg PPE) showed healthy central vein and endothelial lining, with widespread microvesicular fatty changes throughout the acinar zones, reactive Kupffer cells, and dilated sinusoids; (F) G6 (fed with 200-mg/kg PPE) showed microvesicular fatty alterations with intact nuclei in hepatocytes, and dilated sinusoids with increased bile pigments activated Kupffer cells; (G) G7 (treated with metformin hydrochloride) showed well-formed hepatic cords, activated Kupffer cells, and intact endothelial lining of the central vein without thickened walls.
response to glucose. This prolonged suppression may contribute to hyperglycemic conditions. Additionally, Weaver et al. (1978), reported that alloxan caused type 1 diabetes mellitus by destroying β cells of islets of Langerhans.

After administering PPE for 28 days, a noticeable decrease in glucose level was observed on different days (day 2, 8, 15, and 22). To lower glucose levels, a maximum dose of PPE (200 mg/kg) was found to be more effective than lower doses (50, 100, and 150 mg/kg), which was in line with the results of prior studies (Attia et al., 2014, Jain et al., 2012, Khalil, 2004). According to Khalil (2004), PPE effectively reduced post-treatment levels of blood glucose, elevated insulin levels, and increased the number of β cells.

In addition, it was reported that PPE has antioxidant properties of a scavenger of free radicals, and effectively protects β cells of islets of Langerhans from damage (Chidambara Murthy et al., 2002). After 28 days, a substantial increase in bilirubin and CRP values was observed in the positive control G2 group. According to a prior study conducted by Wang et al. (2017), pre- and newly onset diabetes could have elevated bilirubin levels because of oxidative stress and inflammation.

Additionally, a consistently high glucose levels elevate CRP levels (Levy et al., 2004). Compared to the positive control group (G2), a significant decrease was observed in the total bilirubin levels of PPE-administered groups. The results were the same as reported by Tehseen et al. (2022) and Monem et al. (2011). Also, a significant decrease in CRP values was observed, which was in agreement with the findings of Salama et al. (2021). A similarly significant decrease was observed in direct and indirect bilirubin levels. This decrease in bilirubin levels could be due to presence of a bioactive compound (ellagic acid) in PPE (Ríos et al., 2018; Yağmur and Şahin, 2020). According to the findings of Zhang et al. (2017), a considerable increase was observed in the total counts of RBCs, WBCs, granulocytes, lymphocytes, and platelets in untreated diabetic mice. Findings of Khalil (2004) disagreed with the results of our study, which could be the result of various experimental models, feeding regimens, and environmental variables. Even though different doses of PPE tended to reduce hematological parameters, the results were not in the accepted reference range. PPE at a dosage of 200-mg/kg along with metformin hydrochloride significantly normalizes hematological parameters. Moreover, Machado et al. (2002) also concluded in a study that the homeostatic properties of Punica grana tum L. could normalize hematological levels in diabetes.

Similar features were observed in liver histology after comparing PPE-treated groups to the metformin hydrochloride-mediated group in non-diabetic mice. Contrary to the positive control mice (G2), groups that received PPE and metformin hydrochloride showed a dose-dependent improvement. Current investigations concurred with the findings of the studies conducted by Zhang et al., (2023), Faddladdeen and Ojaimi (2019), and Almuttairi RS. 2023. The primary characteristics observed were the central vein with a well-defined shape and regular endothelial lining, absence of perivascular fibrosis, and well-differentiated hepatic cords and nuclei. Studies have shown that the hepatoprotective function of PPE could be due to its phenolic compounds (Faddladdeen and Ojaimi, 2019) and enhancement of enzymatic and nonenzymatic activities (Zhai et al., 2018). According to Khan et al. (2018), PPE has hepatoprotective properties against the toxic effects of carbon tetrachloride in mice liver.

**Conclusion**

The current study was carried out to find the therapeutic potential of PPE against in vivo-induced diabetes and diarrhea in mice. A significant effectiveness of various doses of PPE (50, 100, 150, 200 mg/kg) was discovered in biochemical and hematological parameters in both diabetes and diarrhea. The therapeutic role of PPE was further confirmed with histopathology of the liver and intestine. Restoration of altered parameters was observed in the experimental models of both antidiabetic and anti-diarrheal mice groups, compared to the negative control group. The effectiveness of PPE varied from lower to higher dose. The higher dose (200 mg/kg) was found more effective in normalizing the parameters. The therapeutic restoration of these parameters by PPE confirmed its medicinal potential against diabetes and diarrhea.

**Data Availability Statement**

All the data generated in the study is given in the manuscript.

**Conflicts of Interest**

The authors declared no conflict of interest.

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References


(2022YFS0617) to JW and Southwest Medical University postdoctoral grant (00040177) to WA.
Studying medicinal potential of methanolic pomegranate peel extract


Maglano D.J. and Boyko E.J. 2022. IDF Diabetes Atlas. PMID: 35914061


