Anti-Ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds of pomegranate juice fortified with peel powder or seed homogenate in experimental rats

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ABSTRACT

Natural products are used to develop anti-ulcer drugs with minimal side effects. In the present study pomegranate juice samples fortified with peel powder or seed homogenate were investigated for the treatment of induced peptic ulcer in rats. Forty-two female Albino rats weighing about 150±5 g were divided into two groups. The first group was fed only the basal diet as a negative. The second major group was randomly divided into six groups (six animals each). Group (2) the Positive control group (ve+) received orally (2 ml/kg BW) distilled water/day/rat by epi-gastric tube. Group (2) pretreated orally with 5mg/day (P. juice). Group (3) pretreated orally with (5mg/day) from (1.5 g PPP /100 ml P. Juice). Group (4) pretreated orally with (5mg/day) from (1.5 g PSP /100 ml P. Juice). Group (5) was pretreated orally with (a 5g/ 100 g diet /day) from (PPP). Group (6) received orally (50 mg/kg BW) Antodine® suspended in distilled water. After 7 days, animals were fasted for 24 hrs. Induction of ulcers in The second major group was done on the last day of the experiment.to induce acute gastric mucosal lesion, ibuprofen was treated orally in a dose of 200 mg/kg body weight three times a day at an interval of 8 hrs. Ulcer index, protective index, volume of gastric juice, and pH of gastric juice were evaluated in all groups. In biochemical parameters, Malondialdehyde (MDA), Reduced glutathione (GSH) concentration, Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, Total protein, Albumin, and Alkaline phosphatase (ALP) activity were determined. The results indicated that the macroscopic and lipid peroxidation in the stomach had significant anti-ulcer activity of pomegranate juice, pomegranate juice enriched with peel powder and seed homogenate, peel powder, and Antoine. The anti-ulcer activity was comparable to the positive control. The results also indicated that there were no significant differences in values of physicochemical parameters including pH, titratable acidity, and TSS among the control rats group and fortified juice sample groups, especially at the level of (1.5 g/100 mL) either in peel powder or seed homogenate. The fortification treatments caused significant differences in the total phenolic, antioxidant activity and total flavonoid content among studied samples. Nineteen phenolic compounds had identified with significant variation among the studied samples. Seventeen volatile compounds were detected in the aroma profiles of pomegranate juice and selected supplemented pomegranate juice samples. Fortified pomegranate juice with peel powder recorded an increase of fenchone and a-terpineol to 5.39% and 4.21%, respectively compared to fresh pomegranate juice. The pretreatments with pomegranate and its derivatives did not produce significant changes in the tested biochemical parameters and histopathology in Female rats with Ibuprofen-induced gastric ulcer. It was concluded the fortification of pomegranate juice by peel powder or seed homogenate could be considered a promising gastroprotective and anti-ulcerative agent.

Keywords: Pomegranate juice, By-products, Physicochemical, Antioxidant, Volatile Ibuprofen; peptic ulcer; Lipid peroxidation; Histopathology; rats.
INTRODUCTION

Gastric ulcer is characterized by its damage to the stomach (Koffuor et al., 2013). It is resulting from the consumption of spicy food, alcohol, in addition to stress, gastric surgery, and infection with Helicobacter pylori (Bae et al., 2011). Ibuprofen is a powerful analgesic and anti-inflammatory drug that is used in the initial treatment of rheumatoid arthritis and osteoarthritis however long-term treatment with this drug can induce ulcers (Liu et al., 2016) which is treated with chemical drugs that inhibit gastric acid secretion, antacids, acid neutralizers (Halabiet et al., 2014). However, most of these drugs showed side effects such as joint pain, cardiac arrhythmias, hematopoietic changes, gynecomastia, and systemic alkalosis (Handa et al., 2014). Recently research is focusing on using natural products for treatments with minimal side impacts (Rasoolet et al., 2006).

Using processed agriculture by-products in real food is a promising and fundamental goal of food processing to reduce its environmental impact (Salami et al., 2019). The high nutritional value of pomegranate by-products favors the use of grain reduction in ruminant feed. Pomegranate fruit contains about 50% peel, 40% parchment, and 10% pips which received a great deal of attention for their nutraceutical and antioxidant properties (Jurenka, 2008). Converting the pomegranate into a juice of about 78% peel and 22% seed creates large amounts of by-products and waste (Cam et al., 2014). All pomegranate components have many active compounds, like phenolic compounds (galactic acid), flavonoids (catechins and quercetin), and anthocyanidins (cyanidin’s), with many biological important and have anti-inflammatory, hepatic, antioxidant properties, bacteriostatic and antiulcer (Melgarejo-Sánchez et al., 2021). The antioxidant activity of pomegranate peel and other by-products is higher than that of the juice due to their richness in phenolic compounds, especially tannins and flavonoids and proanthocyanidins (Tzulker et al., 2007). Moreover, pomegranate has antimicrobial, antifungal, and antimutagenic effects (Iqbal et al., 2008). During the processing and storage of pomegranate juice there was a drastic loss and degradation of phenols, resulting in reduced antioxidant activity and a negative impact on the sensory properties of the final product (Saponjac et al., 2016).

Recently, different food products had supplemented with phenolic compounds from vegetables and fruits as well as their by-products to meet the increasing demand for obtaining functional foods with healthy properties (Abid et al., 2017; Kaderides et al., 2019). The low acceptance and consumption of fresh and canned pomegranates are related to low-odor volatile compounds that decrease or disappear during processing. The low aroma concentration represents a major obstacle to the isolation and identification of pomegranate volatile compounds. In fresh fruit, the predominant volatile compounds were limonene, hexanal, and α-terpineol (Melgarejo et al., 2011). On the other hand, esters, particularly ethyl butyrate and isopentyl acetate, and alcohols such as hexanol have been identified by pervaporation as common volatile compounds in pomegranate juice (Raisi et al., 2008). Mphahlele et al. (2016) identified about 10 volatile compounds in pomegranate juice and stated that the main volatile compounds are ester (ethyl acetate) and ketone (3-octane).

Therefore, the current study aims to identify the impact of pomegranate by-products such as peel and seeds in treatment of gastric ulcers induced by Ibuprofen in experimental rats and using its peel and
seeds by-products to fortification natural pomegranate juice in terms of phytochemical analysis, antioxidant activity as well as volatile compounds.

MATERIALS AND METHODS

1. Materials:
- Pomegranate fruits were obtained from the local market, in Cairo, Egypt.
- 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid, gallic acid, Folin-Ciocalteu’s reagent, sodium carbonate, aluminum chloride (AlCl3) were obtained from Sigma-Aldrich.
- Kits used for determining Malondialdehyde (MDA), Reduced glutathione (GSH) concentration, and Serum total antioxidant capacity (TAC) were obtained from Bio-diagnostic Co. Dokki, Egypt.
- Serum ALT, AST, ALP, total proteins, and albumin content kits were purchased from Spinreact, Germany.
- Antonin, Ibuprofen, casein, sucrose, corn oil, fiber (cellulose), mineral mixture, vitamin mixture, choline chloride, D-L methionine, and corn starch were obtained from El-Gomhoreya Co., Cairo, Egypt.
- Female Albino rats weighing about (150±5g) were obtained from Agricultural Research Center, Giza, Egypt.

2. Preparation of pomegranate juice (P. juice)

Fresh pomegranates were washed and stored at 4°C until use. The fruits were peeled by hand without removing the pits. Pomegranate juice was obtained by pressing with a commercial mixer (Braun-Mischer, Germany) and filtered to remove residues. According to Hadipour et al. (2010) the juice was used within one hour after pressing and filtering.

2.1. Preparation of pomegranate peel powder (PPP)

The pomegranates are washed in distilled water and cut by hand to separate the pit and skin. The skin is cut into small pieces with a sharp knife and dried in a forced air dryer at 60 ± 5°C for about 12 hours or until moisture content is about 12-14%. The dried pieces were cooled and pulverized to pass through a 20-mesh sieve, then packed in high-density polyethylene bags, and stored at (25 ± 5 °C) until use according to Jalal et al. (2018).

2.2. Preparation of pomegranate seed powder (PSP)

Pomegranates are washed in distilled water and cut by hand to separate the pit and skin. To extract juice from the pomegranate, the stalks of the pomegranate must be squeezed by hand. The obtained pomegranate seeds (PS) are washed with distilled water to remove the adhering pomegranate pulp and dried in a drying rack with air circulation at 60 ± 5 °C for 6 hours or until a moisture content is ~5-6% Dried seeds should be refrigerated, powdered to pass through a 40-mesh sieve, then packed in high-density polyethylene bags and stored at 25 ± 5 °C until use according to Jalal et al. (2018).

3. Physicochemical properties measurements of pomegranate juice enriched with peel powder and seed homogenate

The physicochemical prameters including pH, titratable acidity (TA), and total soluble solids (TSS) were measured in triplicate according to the Association of Official Analytical Chemists (2019) as follows:

3.1. Determination of pH and titratable acidity

10 mL of each Juice sample was diluted in 10 mL of deionized water to measure the pH value using a pH meter (Hanna pH-meter
Juice samples were subjected to a titration process using 0.01 N NaOH solution and 5 drops of 5 g/100 mL phenolphthalein solution to calculate the total titratable acidity (TTA) as citric acid equivalents (g/L) as follows:

\[
TTA = \text{Dilution factor (10)} \times V(\text{NaOH}) \times 0.01 \times 0.009 \times 100
\]

3.2. Total soluble solids (TSS)

It was determined by using handheld sugar refractometer. TSS was read off the refractometer scale when held close to the eye.

4. Phytochemical analysis of pomegranate juice, peel powder, and seed homogenate

4.1. Preparation of extract

500 ml boiled distilled water was added to 10 g bark powder or 20 ml juice sample. The mixture was left for 5 minutes. The resulting extract was filtered with Whatman #1 filter paper. The extracts were stored at 4°C for further experiments (Naveena et al., 2008).

4.2. Determination of the Total Phenolic Content (TPC)

The total polyphenol content was determined by the Folin-Ciocalteu test (Singleton et al., 1999). 0.1 mg/ml of the extract was added to distilled water, then 5 ml of 10% Folin-Ciocalteu Reagent (FCR) and 4.5 ml Na2CO3 solution (7.5% w/v) were added to 500 µl of the sample. The final solution was stirred for 2 hours in dark, then a absorbance of the mixture was measured at 765 nm. The concentration of total polyphenols was expressed in mg gallic acid equivalents (GAE)/g extract. The test was performed in triplicate and the means ± SD were used.

4.3. Determination of total flavonoid content (TFC)

TFC was measured using a modified aluminum chloride colorimetric test as described by Liu et al. (2008). An aliquot of the diluted sample or the (+ve) catechin standard solution was added to 75 µL of the NaNO2 solution (7%) and were stirred for 6.0 minutes before adding 0.15 mL of AlCl3 (10%). After 5 minutes 0.5 ml NaOH solution (1 M) was added. The final volume was adjusted to 2.5 ml, mixed well, and the absorbance of the mixture was determined at 510 nm. Total flavonoid was expressed in mg rutin equivalent per gram dry residue (mg RE/g) using a rutin calibration curve (range 0-400 µg ml⁻¹). The test was performed in triplicate and the means ± SD were used.

3.4. Total anthocyanin content (TAC)

The TAC of the samples was determined using the differential pH method. Sodium acetate (pH 4.5, 0.4M) and potassium chloride (pH 1.0, 0.025 M) were used as a buffer. Sodium acetate buffer (3.6 mL) was mixed with 0.4 mL of the sample. The absorbance (spectrophotometer, spec. 1601-PC, Shimadzu, Japan) was measured against the blank pad at 510 and 700 nm. The total anthocyanin content was calculated using the following equation:

\[
TAC (\text{mg cyaniding-3-glucoside/100 mL of sample}) = \left( \frac{A \times MW \times DF \times 100}{MA} \right)
\]

Where, \( A = (A_{510} - A_{700}) \text{ pH 1.0} - (A_{510} - A_{700}) \text{ pH 4.5}; \) PM: molecular weight of cyanidin-3-glucoside (449.2); DF: dilution factor (10); AM: molar absorbance of cyanidin-3-glucoside (26,900) (Ravanfar et al., 2015). The test was performed in triplicate and the means ± SD were used.

4. Antioxidant Activities

4.1. Radical scavenging activity (RSA) by DPPH:

To determine the RSA of the samples, 0.1 ml of the sample solution was mixed with 3.9 ml of methanolic DPPH0 (25 mg/l). The absorbance values of the sample were determined at 517 nm after storage at room temperature for 30 minutes. The percentage
Anti-Ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds of pomegranate juice fortified with peel powder or seed homogenate in experimental rats

of inhibition of DPPH® (I%) was determined according to the following equation (Brand-Williams et al., 1995).

\[ I\% = \left( \frac{A_{c} - A_{s}}{A_{c}} \right) \times 100 \]

Where, Ac: absorbance of the blank (with the same chemicals except for the sample). As: sample absorbance. Percent inhibition was then plotted against the respective concentrations. IC50 values were calculated as the extract concentration required to achieve 50% DPPH scavenging activity.

4.2. ABTS Radicalcation Scavenging Activity

The radical scavenging activity of 2,2′-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) was measured according to a standard protocol (Wong-Paz et al., 2015). Stock solutions of 7.0 mM ABTS+ (ammonium salt (2,2′-casino-bis (3-ethylbenzothiazoline sulfone)) and 2.45 mM potassium persulfate were prepared in water and kept in the dark for 12 hours. Equal volumes of the stock solutions were mixed and diluted to an absorbance of 0.70 ± 0.02 at 734 nm to prepare the ABTS+ radical solution. 50 μl of different concentrations (25-500 μg/ml) of each extract or control (50 μl distilled water) were mixed with ABTS+ radical solution (1 ml) and measured immediately and absorbance was measured at 734 nm after 10 minutes. The absorbance of the sample was compared to that of the control. Percent inhibition and IC50 values were then measured and calculated as described for DPPH.

5. Determination of Phenolic compounds by HPLC

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 μl for each of the sample solutions. The column temperature was maintained at 40 °C.

6. Volatile compounds analysis by Gas chromatography-mass spectrometry analysis (GC-MS)

The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. Samples were diluted with hexane (1:19, v/v). The GC was equipped with a DB-624 column (30 m x 320 μm internal diameter and 1.8 μm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 3.0 ml/min at a split 1:20 of, injection volume of 1 μl and the following temperature program: 40 °C for 1 min; rising at 7 °C /min to 250 °C and held for 5 min. The injector and detector were held at 250 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 30-440 and solvent delay 6 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

7. Identification of volatile compounds

The volatile compounds were identified by comparing their retention rates and mass spectra to standards or, in some cases only tentatively, to the NBS 75K Mass Spectral Library search and retention rates. Retention indices were calculated for each compound using a series of homologous C6-C22 n-alkanes (Adams, 2007).
8. Biological experiment

Forty-two female albino rats weighing approximately 150 ± 5 g were acclimatized for one week in an atmosphere of filtered, pathogen-free air, water and a temperature of 20-25°C for 8 weeks, with a 12-hour light/dark cycle and a light cycle (8-20 h) and a relative humidity of 50%. All rats were fed a basal diet. The basal diet was designed to contain 14% casein, 10% sucrose, 4% corn oil, 5% fiber (cellulose), 3.5 percent mineral mixture, 1% vitamin mixture, 0.25 percent choline chloride, 0.3 percent D-L methionine, and 61.95 percent corn starch (Reeves et al., 1993). All the experimental procedures were carried out by international guidelines for the care and use of laboratory animals. The experiment was conducted at Agricultural Research Center, Giza, Egypt.

8.1. Experimental design:

After acclimatization period rats were divided into two groups. The first group (n=6 rats) received only the basic diet as a negative control group (ve-) (Healthy rats). According to the protocol, the second main group was randomly divided into six groups (six animals each) as follows:

Group (2) the Positive control group (ve+) received orally (2 ml/kg BW) distilled water/day/rat via a supra gastric gavage.
Group (3) pretreated orally with pomegranate juice (P. juice) 5mg/day.
Group (4) pretreated orally with (5mg/day) from pomegranate juice with peel powder (1.5 g PPP /100 ml P. Juice).
Group (5) pretreated orally with (5mg/day) from pomegranate juice with seed powder (1.5 g PSP /100 ml P. Juice).
Group (6) was pretreated orally with (5g/100 g diet /day) from (PPP).
Group (7) received orally (50 mg/kg BW) Antodine® suspended in distilled water.

After 7 days, animals were fasted for 24 hrs. Induction of ulcers in animals of groups (2-7) was done on the last day of the experiment.to induce acute gastric mucosal lesion. This was done by giving rats Ibuprofen orally in a dose of 200 mg/kg body weight three times a day at an interval of 8 hrs. Ulcer index, protective index, volume of gastric juice, and pH of gastric juice were evaluated in groups (2-7).

At the end of the experiment, the animals were sacrificed for the collection of stomach samples for further analysis to determine the potential and essential mechanisms involved in the treatment of gastric ulceration, and the blood was collected in a clean dry centrifuge tube, left at room temperature until the clot was formed, completely retracted, and then centrifuged to separate serum by centrifugation at 4000 R.P.M. for 10 minutes at room temperature, followed by storage in a plastic vial (well stoppered) until analysis.

8.3. Assessment of gastric mucosal damage (Ulcer Index, AU, IU):

All rats were sacrificed and their stomachs were tied around the two orifices (cardiac and pyloric sphincters) and injected with 3 ml of distilled water. The gastric juice was then collected in a sterilized tube.

Determination of the ulcer index: The ulcer index was determined with a magnifying glass as described by Bandyopadhyay et al. (2004). The sum of the lesion areas for each stomach was used to calculate the area of ulcer (AU) (Robert et al., 1984) and the IU (ulcer inhibition) was calculated using the following formula:

\[ IU\% = \frac{[(UC\ of\ C– UC\ of\ T)]}{UC\ of\ C} \times 100\% \]

Where, IU stands for ulcer inhibition, T for healing, and C for negative control.

8.4. Measurement of the volume of gastric juice:

The gastric juice of each animal was centrifuged at 3000 rpm for 10 minutes to remove any solid residue. Gastric juice volume was measured with a graduated cylinder and expressed in milliliters (mL).
The pH of the supernatant was then determined (Moore, 1968).

8.5. Determination pH of gastric juice:
PH values were determined according to Debnath et al. (1974).

9. Biochemical investigation
9.1. Oxidative stress evaluation:
The gastric wall was cut into several smaller sections and then homogenized by combination with ice-cold 10% phosphate-buffered homogenate. The clear, non-hemolysed supernatant was then centrifuged at 4°C and 3000 rpm for 15 minutes and used to evaluate the malondialdehyde MDA and glutathione (GSH) (Barham and Trinder (1972). Serum samples were used to measure total antioxidant capacity (TAC).

9.2. Malondialdehyde (MDA) assay:
The major product of lipid peroxidation was measured as given by Ohkawa et al. (1979) based on the formation of a reactive thiobarbituric acid (TBA) product with a pink color where its absorbance was measured at 534 nm and MDA in an acid solution at 95°C for 30 minutes.

9.3. Reduced glutathione (GSH) concentration:
The technique uses glutathione (GSH) to reduce 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to create a yellow product compound. Directly from the decreased chromogen proportional to the GSH level and its absorbance was measured at 405 nm.

9.4. Activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST):
This was determined using the calorimetric method of Reitman and Frankel (1957). The total proteins were calculated according to Gornall et al. (1949) method. Albumin was determined using the method described by Doumas (1971). The alkaline phosphatase (ALP) activity was determined colorimetrically with a spectrophotometer (model DU 4700) at 510 nm according to the method of Belfield and Goldberg (1971).

10. Histopathological studies:
All stomachs were quickly removed, incised along the major curvature, and flushed thoroughly with ice-cold saline. After the identification of gastric ulcers, gastric tissue was taken from the anterior part of the stomach and fixed in 10% formalin according to Bancroft et al. (1996). After 24 hours of fixation and subsequent embedding of paraffin blocks, stomach were cut into 5 μm sections and embedded on a microscope slide and stained with hematoxylin - eosin for histological evaluation.

11. Statistical Analysis
Data obtained from this study were subjected to statistical analysis of variance (ANOVA) according to Snedecor and Cochran (1980) using SPSS software version "20" for Windows. The LSD value (Least Significant Difference) was used to determine the significant difference between the mean values. Data were presented as mean ± SD. Values were considered significant at P< 0.05, otherwise, they were considered insignificant.

RESULTS AND DISCUSSION
1. Effect of peel powder and seed homogenate on physicochemical properties of pomegranate juice
Data in Table (1) indicated that there were no significant differences (P≥0.05) in values of the studied physicochemical parameters including pH, titratable acidity, and TSS among the control and fortified juice samples, especially at the level of (1.5
g/100 mL) either in peel powder or seed homogenate. All the investigated samples recorded low pH nearly about 3.0 which indicates the ability for long time storage from a microbiology standpoint. The results obtained were in contrast to those of Dafny-Yalin et al. (2010) who found significant differences between pomegranate juice samples prepared from peel and carcass homogenate. The Egyptian species of pomegranate used in the present study had a pH, TA, and TSS of 2.98, 12.76, and 15, respectively (Table 1). These results agree with those of Melgarejo et al. (2000) and are similar to those of Cam et al. (2009) who studied Turkish varieties of pomegranate.

There were significant differences in total phenolic content between the investigated samples (Table 1). The level of enriched skin powder (2.0 g/100 ml) had a maximum value (8.92 mg/g) compared to the same level of seed (7.14 mg/g) and control (6.0 mg/g) homogenate samples. A similar trend was observed for the total flavonoid content and antioxidant activity. However, an opposite trend was observed for total anthocyanin content. These results are in agreement with Gil et al., (2000) and Tzulker et al. (2007) who mentioned that pomegranate juice with peel residues had about two-fold higher antioxidant activity in pressed fruit juices and about 20-fold higher antioxidant activity in the whole fruit homogenates than in juices processed from the matrix only. Also, the current results showed that the level of the total phenols in the control and fortified pomegranate juice with the addition of peel powder ranged from 6.25 to 8.92 mg/g (Table 1). The present results are in accordance with Ozay-prancingly et al., (2022) who reported that the total phenolic content was between 6.13 and 8.22 mg/g pomegranate seeds. The total phenolic content of pomegranate juice was higher after fortification with peel powder than after fortification with seed homogenate as compared with control sample. This observation could be due to the high concentration of phenolic compounds in the pomegranate skin compared to other parts, as reported mentioned by Akhtar et al. (2015). Moreover, pomegranate juice treated with peel powder generally had higher antioxidant activity (lower IC50 values) than other treatments, including seed homogenate and control treatment. Increasing the peeling powder concentration increased antioxidant activity. With the DPPH method, all tested concentrations of the washing powder showed statistically significant differences from the control.

In the current investigation using DPPH and ABTS+ methods, the peel powder treatment showed the lowest IC50 values, which indicated that it has the highest antioxidant activity among the treatments followed by the seed homogenate treatment. All tested concentrations of peel powder showed statistically significant differences from the control in terms of DPPH value. A good correlation between antioxidant activity and determined phytochemicals like total phenolic, flavonoids, and anthocyanins were observed.
Table (1). Quality parameters of pomegranate juice enriched with peel powder and seed homogenate

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<td>Peel powder (g/100 mL)</td>
<td>Seed homogenate (g/100 mL)</td>
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<td>±0.19</td>
<td>±0.18</td>
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<td>±0.15b</td>
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<tr>
<td>Total phenols (GAE mg /g)</td>
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<tr>
<td>Total flavonoids (RE m/g)</td>
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<td>10.95</td>
<td>11.08</td>
<td>11.27</td>
<td>10.52</td>
<td>10.86</td>
<td>10.95</td>
</tr>
<tr>
<td>(0.12)</td>
<td>±0.05a</td>
<td>±0.15a</td>
<td>±0.13c</td>
<td>±0.09</td>
<td>±0.12a</td>
<td>±0.09</td>
<td>±0.18b</td>
<td>±0.13c</td>
</tr>
<tr>
<td>Total anthocyanins (mg/g)</td>
<td>19.85±</td>
<td>22.34</td>
<td>22.98</td>
<td>23.15</td>
<td>23.85</td>
<td>20.17</td>
<td>20.86</td>
<td>21.15</td>
</tr>
<tr>
<td>(0.24)</td>
<td>±0.18a</td>
<td>±0.12a</td>
<td>±0.12b</td>
<td>±0.13b</td>
<td>±0.09b</td>
<td>±0.16c</td>
<td>±0.14d</td>
<td>±0.19d</td>
</tr>
<tr>
<td>Antioxidant activity (IC50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH0</td>
<td>75.6±</td>
<td>27.8±</td>
<td>19.3±</td>
<td>18.5±</td>
<td>18.3±</td>
<td>51.2±</td>
<td>48.7±</td>
<td>45.2±</td>
</tr>
<tr>
<td>(0.02)</td>
<td>0.07a</td>
<td>0.12a</td>
<td>0.03a</td>
<td>0.15a</td>
<td>0.18</td>
<td>0.24</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>ABST+</td>
<td>43.8±</td>
<td>35.4±</td>
<td>30.4±</td>
<td>27.2±</td>
<td>26.9±</td>
<td>39.5±</td>
<td>34.2±</td>
<td>33.8±</td>
</tr>
<tr>
<td>(0.08)</td>
<td>±0.21a</td>
<td>±0.16</td>
<td>±0.05</td>
<td>±0.18</td>
<td>±0.12</td>
<td>±0.15a</td>
<td>±0.19</td>
<td>±0.08</td>
</tr>
</tbody>
</table>

Values are the mean of 3 replications (± standard deviation). TA: titratable acidity; TSS: total soluble solids; Values followed by the same letter, within the same row, were not statistically different at P ≤ 0.05.

2. Determination of Phenolic compounds by HPLC

Results of analysis of phenolic compounds in different parts of pomegranate including fresh juice, peel, and seed homogenate are shown in Table (2). It was obvious that a total of 19 phenolic compounds with significant variability were identified in the samples tested. The main phenolic compounds in pomegranate juice were chlorogenic acid, gallic acid, and catechin with concentrations of 14.87, 14.52, and 11.61 (mg/ml), respectively. In the bark powder, gallic acid, ellagic acid, and catechin were the dominant phenolic compounds at concentrations of 7.772, 1.761, and 1.546 (mg/g), respectively. The current data agree well with those of El-Hadary and Ramadan (2019).

The phenol profile and total concentration depend on the different parts of the pomegranate and the cultivars (Singh et al., 2018; Talekar et al., 2018). For example, the Tunisian and Spanish varieties contain about 7.3-16mg/ml. Seed homogenate contained fewer phenolic compounds than fresh juice and bark powder (Table 2). The presence of phenolic compounds, particularly ellagic and gallic acid, confers biological activity on pomegranates and their products and by-products, e.g. B. Antioxidant, antibacterial, anticancer, antiatherosclerotic and healing effects (Adhami and Mukhtar, 2006; Dahham et al., 2010; Rajan and others, 2011).

In the current study the estimated values of gallic and ellagic acid...
concentrations were higher than those reported by Yan et al., (2017) and Li et al., (2015) who reported found that gallic acid and ellagic acid had concentrations of 2.59 and 2.83 mg/g, respectively, in Chinese pomegranate bark extract.

Table (2). Phenolic composition of pomegranate juice, peel, and seed

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Juice (mg/ml)</th>
<th>Peel (mg/g)</th>
<th>Seed (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>14.52</td>
<td>7.772</td>
<td>0.087</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>14.87</td>
<td>0.942</td>
<td>0.055</td>
</tr>
<tr>
<td>Catechin</td>
<td>11.61</td>
<td>1.546</td>
<td>0.066</td>
</tr>
<tr>
<td>Methyl gallate</td>
<td>3.44</td>
<td>0.092</td>
<td>0.002</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>1.22</td>
<td>0.051</td>
<td>0.003</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>1.45</td>
<td>0.044</td>
<td>0.007</td>
</tr>
<tr>
<td>Pyro catechol</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>Rutin</td>
<td>1.14</td>
<td>N.D</td>
<td>0.002</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>1.97</td>
<td>1.761</td>
<td>0.005</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>1.73</td>
<td>N.D</td>
<td>0.006</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.28</td>
<td>0.013</td>
<td>N.D</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.15</td>
<td>0.021</td>
<td>N.D</td>
</tr>
<tr>
<td>Naringenin</td>
<td>0.75</td>
<td>0.060</td>
<td>0.002</td>
</tr>
<tr>
<td>Daidzein</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>Quercetin</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>N.D</td>
<td>0.001</td>
<td>N.D</td>
</tr>
<tr>
<td>Apigenin</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td><strong>Total phenolic</strong></td>
<td><strong>0.053</strong></td>
<td><strong>12.30</strong></td>
<td><strong>0.235</strong></td>
</tr>
</tbody>
</table>

N.D: Not detected

3. Effect of peel powder and seed homogenate on volatile compounds in fresh and selected supplemented pomegranate juice samples

Analysis of volatile compounds in fresh and selected samples of pomegranate juice supplemented based on physicochemical and phytochemical properties and antioxidant activity by GC-MS revealed the presence of hexanal, limonene, and b-myrcene as the main volatile components in freshly prepared pomegranate juice (Table 3). The main problems in accepting pomegranates or prepared juices are the low aromatic flavor, which is also significantly lost during processing (Calin-Sanchez et al., 2011; Melgarejo et al., 2011). Thus, only 17 volatile compounds were identified in this study. The identified volatile compounds can be divided into different chemical groups; monoterpenes (6); monoterpenoids (3); aldehydes (3); esters (3) and alcohols (2).

The results obtained are in agreement with Vazquez-Araujo et al. (2011) who found benzene and listed similar chemical groups and terpene derivatives as the major volatile compounds in fourteen freshly made and commercial pomegranate juices depending on the type of juice.

The data from the present study showed that the pomegranate juice samples contained C6 aldehydes such as hexanal and alcohols (such as 1-hexanol) at significant levels, particularly in the post-treatment where 19.52% hexanal was found, followed by a peel juice addition which was 8.27% % and the lowest hexanal concentration was observed in the seed homogenate enriched juice (0.96%) (Table 3). The current results were in accordance to Cadwallader et al. (2010) and Melgarejo et al. (2011) who noted a decrease in green aroma during storage. The main monoterpenoids identified
in this study were fenchone (4.19%) in fresh pomegranate juice and α-terpineol (3.17%) in seed homogenate-enriched juice. Vazquez-Araujo et al. (2011) stated that α-terpineol - floral/lily scent - is the most important volatile component of pomegranate juice. This volatile compound plays an important role in the acceptance of pomegranate juice with various berries (Vazquez-Araújo et al., 2010).

Data in Table (3) showed that the fortification treatment of pomegranate juice with peel powder or seed homogenate leads to an increase in α-Pinene from 6.12% in the control sample to 9.35% and 8.34% in the case of fortification of peel powder and seed homogenate, respectively. A similar trend was found in the following monoterpenes; β-Pinene, β-Myrcene, and γ-Terpinene. The supplemented and fresh pomegranate juice had three monoterpenoids; fenchone, camphor, and α-terpineol (Table 3) with concentrations of 4.19%, 5.74%, and 2.63%, respectively in the fresh sample. While, fortified pomegranate juice with peel powder recorded an increase of fenchone and α-terpineol to 5.39% and 4.21% respectively compared to fresh pomegranate juice. Hexanal was the most predominant aldehyde in pomegranate juice with a concentration of 19.52% followed by octanal (8.61%) and nonanal (5.76%). Generally, all the aldehydes including hexanal, octanal, and nonanal had decreased after fortification treatment compared to fresh pomegranate juice. All the esters and alcohols had increased after fortification treatment especially ethyl acetate which recorded 10.34% after the addition of seed homogenate compared to the fresh sample (4.72%).

Table (3). Volatile composition of pomegranate juices

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>KIs a</th>
<th>Control</th>
<th>Supplemented Juice</th>
<th>Identification method c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Peep</td>
<td>Seed</td>
<td></td>
</tr>
<tr>
<td>Monoterpenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Pinene</td>
<td>859</td>
<td>6.12</td>
<td>9.35</td>
<td>8.34</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>931</td>
<td>4.79</td>
<td>6.82</td>
<td>7.95</td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>985</td>
<td>8.31</td>
<td>11.65</td>
<td>7.36</td>
</tr>
<tr>
<td>Limonene</td>
<td>1031</td>
<td>9.62</td>
<td>13.82</td>
<td>14.02</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>1057</td>
<td>2.58</td>
<td>5.71</td>
<td>5.18</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>1416</td>
<td>0.67</td>
<td>3.28</td>
<td>6.97</td>
</tr>
<tr>
<td>Monoterpenoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenchone</td>
<td>1080</td>
<td>4.19</td>
<td>5.39</td>
<td>3.12</td>
</tr>
<tr>
<td>Camphor</td>
<td>1139</td>
<td>5.74</td>
<td>2.67</td>
<td>2.95</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>1186</td>
<td>2.63</td>
<td>4.21</td>
<td>3.17</td>
</tr>
<tr>
<td>Aldehydes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexanal</td>
<td>791</td>
<td>19.52</td>
<td>8.27</td>
<td>0.96</td>
</tr>
<tr>
<td>Octanal</td>
<td>998</td>
<td>8.61</td>
<td>4.82</td>
<td>1.27</td>
</tr>
<tr>
<td>Nonanal</td>
<td>1100</td>
<td>5.76</td>
<td>0.18</td>
<td>0.03</td>
</tr>
<tr>
<td>Esters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>648</td>
<td>4.72</td>
<td>3.95</td>
<td>10.34</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>995</td>
<td>6.49</td>
<td>5.74</td>
<td>9.18</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>1997</td>
<td>1.79</td>
<td>0.81</td>
<td>6.12</td>
</tr>
<tr>
<td>Alcohols</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Hexen-1-ol</td>
<td>856</td>
<td>3.62</td>
<td>4.85</td>
<td>7.25</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>876</td>
<td>4.38</td>
<td>6.94</td>
<td>5.34</td>
</tr>
</tbody>
</table>

KIs: Kovat index; Values are expressed as relative area percentage to the total identified volatile compounds. Compounds identified by GC-MS (MS) and/or by comparison of MS and RI of standard compound run under similar conditions.
Table (4) shows the degree of damage to the gastric mucosa (mm$^2$) in different groups. The positive control group (ve+ control) had the highest degree of gastric mucosal damage compared to the control group (9.21 ± 0.52 mm$^2$). The increase in ulcer index and lack of ulcer inhibition after treatment with ibuprofen clearly explain the compromised integrity of mucosal protection and correlate with the damage caused by the administration of ibuprofen (200 mg/kg) to induce gastric ulceration. This is consistent with the results of Narayan et al. (2005) and Liu et al. (2016). Pretreatment with pomegranate juice, pomegranate juice supplemented with bark powder and seed homogenate, bark powder, and Antoine provided significant protection ($p \leq 0.05$) against ibuprofen-induced gastric ulcers in experimental rats. Group 3 (P. juice) and Group 4 (PPP juice + P.) showed a significant reduction in the lesion area of the gastric mucosa with values of (2.72 ± 0.64 and 2.05 ± 0.25 mm$^2$) and exhibit a low percentage of protection from gastric mucosal damage (70.42 and 77.75%, respectively). While group 6 (PPP) had the smallest gastric mucosal lesion area (0.63 ± 0.6 mm$^2$) and showed a high percentage of protection from gastric mucosal damage (93.08%) compared to other treatment groups. Group 5 (PSP + P. Juice) and Group 7 (Antodine) also showed a significant reduction in gastric mucosal lesion area with values of (0.65 ± 0.44 and 0.87 ± 0.24mm$^2$ respectively) and display a protection percentage with values (92.92% and 90) or 50%. The current results were consistent with Abdulzahra and Salih (2022).

The anti-ulcer effect of PP may be due to its action on protective factors in the gastric mucosa and increasing dissolved mucus and reducing desquamation of mucus cells (Dorababu et al., 2004). The antiulcer agent PP has also been attributed to pomegranate, which is rich in ellagic acid, ellagitannins (including punicalagin) (Shukla et al., 2008), alkaloids such as pelletierines, pseudopelletierins (Kirtikar and Basu, 2000) and in punicic acid, in flavonoids, in anthocyanidins, anthocyanins and flavonolsestrogens, and flavones (Jurenka, 2008).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gastric Mucosal Injury Area (mm$^2$)</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Control (ve-)</td>
<td>0±.0$^a$</td>
<td>-</td>
</tr>
<tr>
<td>Group 2: Control (ve+)</td>
<td>9.21±.52$^c$</td>
<td>-</td>
</tr>
<tr>
<td>Group 3 (P. juice)</td>
<td>2.72±.64$^d$</td>
<td>70.42</td>
</tr>
<tr>
<td>Group 4: (PPP + P. Juice )</td>
<td>2.05±.25$^c$</td>
<td>77.75</td>
</tr>
<tr>
<td>Group 5: (PSP + P. Juice )</td>
<td>0.65±.44$^b$</td>
<td>92.92</td>
</tr>
<tr>
<td>Group 6: (PPP)</td>
<td>0.63±.6$^c$</td>
<td>93.08</td>
</tr>
<tr>
<td>Group 7: (Antodine)</td>
<td>0.87±.24$^b$</td>
<td>90.50</td>
</tr>
</tbody>
</table>

Table 4: Effect of pomegranate juice, pomegranate juice enriched with peel powder and seed homogenate, peel powder, and Antodine on gastric lesion surface induced by Ibuprofen in Rats.

Data are presented as means ± SDM ($n=6$). a, b, c and d: Means with different letter among groups in the same column are significantly different($P \leq 0.05$): P: pomegranate, PPP: pomegranate peel powder and PSP: peel powder and seed homogenate.

Biochemical assessment of total gastric acidity and mucosal integrity is commonly used to determine the state of the stomach after exposure to pharmacological drugs. The acidity level and volume of gastric secretions in the stomach are determined by...
their pH. A low pH value indicates a decrease in hydrogen ion concentration in gastric juice. Experimentally, this has been linked to the pathogenesis of ulcers and gastric damage. Table (5) shows the pH and gastric acid levels of the different groups. The negative control group showed the highest pH (1.8 ± 0.44) and the lowest gastric acidity (4.98 ± 0.31), while the positive control group showed the lowest pH (0.5 ± 0.0) and the highest gastric juice content (6.18 ± 0.50). The large volume of gastric secretion and the low pH of gastric juice cause severe damage to the gastric mucosa (Yi et al., 2015). To maintain gastric integrity, gastric mucosal lesions are commonly treated with antacids, proton pump inhibitors, histamine H2-receptors, prostaglandins, cytoprotective agents, agents that suppress gastric secretion, and antibiotics to reduce gastric acid secretion and increase gastric acid production. Mucus, increase bicarbonate production, and provide a defense mechanism to protect surface epithelial cells (Ateufack et al., 2015). Group (3) ((P. juice) ) had a gastric juice content of (5.82 ± 0.40ml) and PH (0.6 ± 0.22), while the group 4 (PPP + P. Juice) had gastric juice and PH (5.72 ± 0.20 and 6 ± 0.22ml, respectively). The Juice + Seeds group (5) showed a 5.12 ± 0.37ml lower gastric juice level than the Juice and Juice + Peel groups. The Antodine group (6) and the Peel group (7) showed similar gastric juice values (5.1 ± 0.62 and 5.0 ± 0.54ml, respectively).In addition, the table showed that the juice group and the (PPP + P. Juice) group had similar pH values (0.8 ± 0.44). With the same trend, the group (PSP + P.Juice) and the Antodine group showed pH values of (0.6 ± 0.22). The results suggest that the treatment may cause an impact on pH and gastric acid levels in the induced groups compared to the control group. The maximum healing effect was in bark powder, pomegranate juice enriched with bark powder, and seed groups. Similar results were obtained by Shekhar et al. (2017) who indicated that pomegranate's potent antiulcer effects. Pomegranate induced increased mucus production, particularly in ulcerated rats. Mucus serves as the first line of defense against ulcers (Hussein et al., 2014). Gautam (2012) reported that the administration of an aqueous extract of pomegranate peel to rats with aspirin-induced ulcers produced a significant improvement in ulcers compared to the peel and seeds of this fruit. Pomegranates are a popular functional food that has antioxidant and anti-inflammatory effects in vivo and in vitro (Kim et al., 2017). Ellagic acid, a phenolic lactone compound, is the main product in vivo hydrolysis of pomegranate polyphenols and has anti-inflammatory, antioxidant, hepatoprotective, and antimutagenic effects (Farzaei et al., 2015).

**Table 5. Effect of pomegranate juice, pomegranate juice enriched with peel powder and seed homogenate, peel powder, and Antodine on volume of gastric juice (mL) and pH of gastric juice induced by Ibuprofen in rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Volume of gastric juice (mL)</th>
<th>pH of gastric juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ve-)</td>
<td>4.98±.31a</td>
<td>1.8±.44c</td>
</tr>
<tr>
<td>Control (ve+)</td>
<td>6.18±.50b</td>
<td>0.5±.0a</td>
</tr>
<tr>
<td>Group 3 (P. juice)</td>
<td>5.82±.40b</td>
<td>0.6±.22ab</td>
</tr>
<tr>
<td>group 4 (PPP + P. Juice )</td>
<td>5.72±.20b</td>
<td>0.6±.22ab</td>
</tr>
<tr>
<td>group 5(PSP +P. Juice )</td>
<td>5.12±.37a</td>
<td>0.8±.44ab</td>
</tr>
<tr>
<td>group 6 (PPP)</td>
<td>5.1±.62a</td>
<td>1.1±.65b</td>
</tr>
<tr>
<td>group 7 (Antodine)</td>
<td>5.0±.54a</td>
<td>0.8±.44ab</td>
</tr>
</tbody>
</table>

Data are presented as means ± SDM (n=6).a, b, c and d: Means with different letter among groups in the same column are significantly different (P ≤ 0.05) P: pomegranate, PPP: pomegranate peel powder and PSP : peel powder and seed homogenate.
4. Biochemical Analysis:

The generation of reactive oxygen species (ROS) and oxidative stress damage is a crucial step in the pathogenesis of gastric ulcers. Oxidative stress is a state that has been shown to change a variety of physiological reactions, and it has a role in both the psychological and pathological onset of stomach ulcers. Results of the present study showed that ibuprofen (IBU) intake significantly encouraged oxidative stress in the stomach of rats when compared to the stomach of rats in other groups. Data in Table (6) revealed significant elevation of gastric malondialdehyde MDA in the group exposed to ibuprofen alone when compared to the control group. Also pretreatment with pomegranate juice and/or pomegranate seeds showed a significant reduction in malondialdehyde MDA levels. In addition, the ibuprofen-exposed group showed significant depletion of gastric GSH and serum TAC when compared to the control group, while the pretreatment with pomegranate juice and/or pomegranate seeds showed significant elevation in gastric GSH and serum TAC when compared to ibuprofen exposed group.

Reactive oxygen species (ROS) are excessively generated by exposure to ultraviolet (UV) light, smoking, alcohol consumption, use of NSAIDs, and many other exogenous substances (Pizzino et al., 2017). In addition, the continuous production and elimination of ROS ensures a dynamic balance of the oxidative-antioxidant system, and an imbalance of this system is one of the main causes of gastric ulcers (Liu et al., 2021). The gastric damage caused by ibuprofen is not yet known, but it is believed that the main cause is cellular damage from reactive oxygen species (ROS). Ibuprofen inhibited the activity of the antioxidant enzyme, resulting in a reduction in the scavenging activity of ROS (Liu et al., 2016). Consistent with the results documented in the present study, the ibuprofen-exposed group showed a markedly impaired gastric redox state with a significant increase in gastric malondialdehyde (MDA) concentration and a decrease in gastric glutathione (GSH) and antioxidant levels Capacity. Total serum (CT) were significantly reduced compared to the control group. The balance between the inside and outside of cells is disrupted, leading to cell damage. The phospholipids of the primary cell membrane were oxidized, the structural integrity of the membrane was compromised, and the fluidity and permeability of the membrane increased. Thus, ROS is essential for ibuprofen-induced gastric ulcers (Liu et al., 2016). On the other hand, excess ROS can induce peroxidation of lipids, proteins, nucleic acids, and other biological components. Peroxidation, accompanied by elevated malondialdehyde MDA levels, can severely damage the mucosal surface of gastric tissues, ultimately leading to tissue and organ damage and rupture (Pizzino et al., 2017).

In addition, exogenous thiol compounds have been shown to protect the stomach from ethanol-induced necrotic changes. The gastric mucosa is rich in glutathione, an endogenous thiol (GSH) (Robert et al., 1984). In the glandular tissue of the stomach, GSH concentrations are much higher, potentially providing additional protection from gastric acid (Pizzino et al., 2017). Therefore, we hypothesized that the decreased gastric GSH content and increased MDA content due to excessive ROS production might directly contribute to the pathogenesis of ibuprofen-induced gastric ulcer disease.

In recent years, various antioxidants have been studied for their actual or potential antioxidant properties. A highly nutritious fruit, pomegranate contains a
Anti-Ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds of pomegranate juice fortified with peel powder or seed homogenate in experimental rats

A variety of health-promoting compounds, including polyphenols, alkaloids, tannins and flavonoids, vitamin C, and minerals. These chemicals have several healing effects (Elfalleh et al., 2011). The data presented in the present study showed a significant sedative effect of pomegranate juice, pomegranate seeds, and pomegranate peel. Significantly improves oxidative stress parameters (serum TAC and gastric MDA, GSH). Of particular note is the antioxidant and reparative action of punicalagin (PCG), found in pomegranate juice, which restores nitric oxide and mucin levels, as well as inhibiting oxidative stress and mucosal inflammation via the NF-kB pathway (Katary and Salahuddin, 2017). The potential benefits of pomegranate could be due to its various metabolites. The antioxidant properties of pomegranate are mainly due to the high concentration of polyphenols in the pomegranate fruit, such as B. ellagitannins and hydrolyzable tannins (Gil et al., 2000). Pomegranate extract can suppress COX-1 and COX-2 enzymes as well as IL-1 activity (Tao et al., 1998). In agreement with the results of the present study, Abd el-Rady et al. (2021) found that pomegranate peel extract and its nanoparticles were able to increase serum TAC and MDA in gastrointestinal gastric ulcer disease in men.

Table (6): Effect of pomegranate juice, pomegranate juice enriched with peel powder and seed homogenate, peel powder, and Antodine on oxidative stress parameters (TAC, MDA, and GSH) in Ibubrufen-induced gastric ulcer in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Oxidative stress parameters</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TAC (Mm/l)</td>
</tr>
<tr>
<td>Control (ve-)</td>
<td>2.08 ± 0.24 a</td>
</tr>
<tr>
<td>Control (ve+)</td>
<td>0.45 ± 0.11 b</td>
</tr>
<tr>
<td>Group 3 (P. juice)</td>
<td>1.10 ± 0.14 c</td>
</tr>
<tr>
<td>group 4 (PPP + P. Juice)</td>
<td>1.45 ± 0.10 d,e</td>
</tr>
<tr>
<td>group 5(PSP + P. Juice)</td>
<td>1.18 ± 0.18 c,d,e</td>
</tr>
<tr>
<td>group 6 (PPP)</td>
<td>1.05 ± 0.17 e</td>
</tr>
<tr>
<td>group 7 (Antodine)</td>
<td>1.72 ± 0.13 c</td>
</tr>
</tbody>
</table>

Data are presented as means ± SDM (n=6). a, b, c and d: Means with different letter among groups in the same column are significantly different(P ≤ 0.05): pomegranate, PPP: pomegranate peel powder, PSP : peel powder and seed homogenate ,MDA: malondialdehyde; GSH: reduced glutathione and TAC: total antioxidant capacity.

Table (7) presents the effect of pomegranate juice, pomegranate juice enriched with peel powder and seed homogenate, peel powder, and Antodine on different biochemical parameters (AST, ALT, ALP, total proteins, and albumin) in male rats with Ibuprofen-induced gastric ulcer. Based on the table (7), we can observe the following. There are no significant differences in AST, ALT, ALP, total proteins, and albumin levels between the control (ve-) and control (ve+) groups. The treated groups show similar levels of AST, ALT, ALP, total proteins, and albumin to the control groups. Based on the results of this table, the pre-treatments with pomegranate and its derivatives did not produce significant changes in the tested biochemical parameters in male rats with Ibuprofen-induced gastric ulcer. Our results agree with Idorenyin et al., (2022) who revealed that there was no significant statistical difference.
in total bilirubin, ALT and AST when compared to the control. This suggests that NSAIDs may have no significant effects on the liver for this regimen. NSAIDs may have harmful effect on the cytoarchitecture of the liver which can lead to liver damage especially when given in high doses and in combination, but however it had no effect on liver function. Though reports of liver toxicity with ibuprofen are rare, subacute hepatic failure requiring orthotopic liver transplant has been reported in a 59-year-old female taking 600mg of ibuprofen (Rodriguez-Gonzalez et al., 2002).

Traversa et al., (2003) in their cohort study that recruited patients receiving various NSAIDs confirmed that ibuprofen has a low liver toxicity rate: only two out of 26 patients that took the NSAIDs showed ibuprofen- associated liver injury. This is probably because ibuprofen is characterized by a high safety profile and very low toxicity incidence and this is based on the fact that ibuprofen has short plasma half-life and does not form pathological metabolites (Vangiessen and Kaisser, 2015). The mechanism of this NSAIDs hepatotoxicity involves alteration of covalent protein by reactive metabolites (Schleiff et al., 2021). This supports recent reports that drugs like NSAIDs actually induce hepatotoxicity which results in reduction of the liver tissue (Sriuttha, Sirichanchuen and Permsuwan, 2018) and (Aithal, 2018).

Table 7. Effect of pomegranate juice, pomegranate juice enriched with peel powder and seed homogenate, peel powder, and Antodine on different biochemical parameters in Ibuprofen-induced gastric ulcer in female rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST (U/L)</td>
<td>44.80 ± 3.70 a</td>
</tr>
<tr>
<td></td>
<td>ALT (U/L)</td>
<td>30.00 ± 3.80 a</td>
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<tr>
<td></td>
<td>ALP (U/L)</td>
<td>139.00 ± 7.87 a</td>
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<tr>
<td></td>
<td>TOTAL PROTEINS (g/dl)</td>
<td>6.86 ± 0.55 a</td>
</tr>
<tr>
<td></td>
<td>ALBUMIN (g/dl)</td>
<td>4.73 ± 0.50 a</td>
</tr>
<tr>
<td>Control (ve-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (ve+)</td>
<td></td>
<td></td>
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<tr>
<td>Group 3 (P. juice)</td>
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<td></td>
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<tr>
<td>group 4 (PPP + P. Juice)</td>
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<td>group 5 (PSP + P. Juice)</td>
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<td></td>
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<tr>
<td>group 6 (PPP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>group 7 (Antodine)</td>
<td></td>
<td></td>
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</tbody>
</table>

Data expressed as Mean ± SD. (N=5/group), a; b; c means having different superscript letters in the same column differ significantly at p≤ 0.05. AST, aspartate amino transferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

5.1 Histopathological Examination of the stomach:

The stomach is in a protected anatomical position in the abdominal cavity and can move within certain limits, so it is not easily injured by external force (Zhao et al., 2013). Damage to the lining of the stomach is common and is caused by a variety of factors, including chemical factors such as smoking, drinking strong tea or coffee, and drugs that stimulate the lining of the stomach, such as aspirin and indomethacin, physical factors such as cold or heat, food undigested food, bacteria or their toxins (Zhao et al., 2014). Ibuprofen-induced gastric injury (IBU) can be defined as bleeding, edema, inflammatory infiltration, and epithelial cell loss that can be observed on microscopic examination (Uthman, 2020).

Stomach sections of rats in different experimental groups in Figures (1 & 2). Microscopically, the stomachs of the control rats (-ve) showed normal histoarchitecture of the gastric layers (Fig.
1). On the other hand, in the stomach of rats of the gastric ulcer group (control + ve), histopathological changes characterized by focal necrosis of gastric mucosa associated with infiltration of mucosal and submucosal inflammatory cells and submucosal blood vessel occlusion and edema were detected (arrows) (Fig. 1). Studies have shown that aggressive factors associated with continued use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, aspirin, indomethacin, naproxen, piroxicam, fenoprofen, salsalate, explosion, etc. erode and damage the epithelium of the gastric mucosa (Wallace., 2000). Ibuprofen-induced gastric injury (IBU) can be defined as bleeding, edema, inflammatory infiltration, and epithelial cell loss visible on microscopic examination (Uthman & University, 2020). Gastric sections from IBU-treated rats showed severe degenerative architecture indicative of complete submucosal ulceration which was consistent with previous reports (Adebayo-Gege et al., 2018). Studies suggest that ibuprofen can induce apoptosis in gastric mucosal cells due to increased leukocyte infiltration into the gastric mucosa and subsequent ROS production (Golbabapour et al., 2013). However, the examined sections from pomegranate juice Group (3) (5 ml) described submucosal edema associated with inflammatory cells infiltration and a few strands of fibroblasts proliferation (arrows) (Fig. 2). Furthermore, some examined stomach of rats from pomegranate (Juice + Peel) group (4) showed slight submucosal edema associated with few inflammatory cell infiltrations, whereas, other sections revealed submucosal inflammatory cells infiltration and submucosal fibroblasts proliferation (arrows) (Fig. 2). Meanwhile, stomach of rats from pomegranate (Juice + Seed) group (5) manifested no histopathological alterations except few submucosal inflammatory cells infiltration (Figs. 2). Moreover, sections from pomegranate Peel group (6) exhibited no histopathological alterations (Figs. 2). Similarly the stomach of rats from the Antodine group (7) revealed no histopathological changes except slight submucosal edema was observed in some sections (Figs. 2). The current histopathological results showed a significant improvement in ibuprofen-induced glandular tissue erosion in the pomegranate peel group. A similar result was reported by Colombo et al. (2013) who showed that pomegranate peel hydroalcoholic extracts significantly reduced mucosal lesions on day 6 of treatment.
Anti-Ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds of pomegranate juice fortified with peel powder or seed homogenate in experimental rats

Fig (1). Photomicrograph of sections of stomach for control (ve-) group and control (ve+) group, stained with H & E, X 400.

Fig (2). Photomicrograph of Sections of stomach for different rats groups stained with H & E, X 400.
CONCLUSION

Generally, the mitigation of stomach ibuprofen insults by oral therapy of pomegranate juice, pomegranate juice enriched with peel powder and seed homogenate and peel powder is suggestive of their excellent gastroprotective, anti-inflammatory and antioxidative potentials in Wistar rat models. The study results, though substantial, are by no means exhaustive as some limitations were encountered. Therefore, it was recommend that further advanced investigation of pomegranate juice enriched with peel powder and seed homogenate and peel powder is sorely needed.

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Anti-Ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds of pomegranate juice fortified with peel powder or seed homogenate in experimental rats


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Anti-Ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds of pomegranate juice fortified with peel powder or seed homogenate in experimental rats

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Anti-Ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds of pomegranate juice fortified with peel powder or seed homogenate in experimental rats

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Uthman, Z.S. (2020). Anti-Ulcerative effect of aqueous extract of Cucumis melo non-steroidal anti-inflammatory drugs (NSAIDS) induced gastric ulceration in male wistar rats. Project Baze University, Abuja, Department of Human Physiology, Faculty of Basic Medical sciences


Anti-Ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds of pomegranate juice fortified with peel powder or seed homogenate in experimental rats

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The study aimed at investigating the anti-ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds of pomegranate juice fortified with peel powder or seed homogenate in experimental rats. The results showed that pomegranate juice fortified with peel powder or seed homogenate had anti-ulcer activities, physicochemical properties, antioxidant activity, and volatile compounds. The juice also had a high antioxidant activity and volatile compounds.

The results indicated that the juice fortified with peel powder or seed homogenate had a high antioxidant activity and volatile compounds. The juice also had a high antioxidant activity and volatile compounds.

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