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Original Research Article

Ameliorative Effect of Pomegranate Molasses on Phenylhydrazine-Induced Anemia in Rats

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ABSTRACT

Pomegranate molasses and peels contain minerals, antioxidants and several vitamins. The aim of this study was to investigate the effect of pomegranate molasses to protect against phenylhydrazine induced anemia in rats. 40 male rats were divided into four equal groups; a control group, Pomegranate molasses treated group, Phenylhydrazine (PHZ) treated group, Pomegranate molasses + Phenylhydrazine treated group. Hematology, hemoglobin electrophoresis, G6PD, Iron, and erythropoietin were evaluated. Spleen was obtained for histopathological examination. The results revealed a significant increase in reticulocytes, osmotic fragility, iron and erythropoietin with a significant decrease in Glucose-6-Phosphate Dehydrogenase in Phenylhydrazine group, compared with Control group. Co-administration of Pomegranate molasses with Phenylhydrazine showed significant decrease in reticulocytes, osmotic fragility, iron and erythropoietin and significant increase in Glucose-6-Phosphate Dehydrogenase compared with Phenylhydrazine group. It could be concluded that pomegranate molasses could improve hematological parameters and had anti-inflammatory effect on spleen.

***Keywords*:** pomegranate molasses, hematology, spleen, Phenylhydrazine.

Introduction

By causing red blood cells to be destroyed by oxidative stress and a number of other cellular changes, phenylhydrazine caused hemolytic anemia. The PHZ-induced toxic anemia can be used as a model to study the pathophysiology of hemolytic anemia, the effects of anemia on other physiological systems, and the emergence of associated illnesses. [1]

Due to the intrinsic antioxidant capacity of the pomegranate fruit, pomegranate molasses (PM), which is used as a condiment, is believed to have substantial impacts on atherosclerosis, cholesterol levels, and cancer prevention. Additionally, the portion of the fruit utilized and the cultivation and climatic circumstances during fruit maturity and ripening, all affect the antioxidant activity of pomegranate molasses.[2]

PHZ poisoning results in erythrocyte oxidative damage, which leads to hemolytic anemia with involvement of the spleen and liver. It results in progressive haematological changes, inflammatory mediators, and enhanced red cell apoptosis because it destroys the cell membrane. PHZ was known to produce lipid peroxidation in the spleen, kidney, and liver. Additionally, after PHZ intoxication, it has been documented that oxidative stress-induced hemodynamic disruption, vascular dysfunction, and electrolyte derangement take place. [3]

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Therefore, the aim of this study was to study the protective effect of Pomegranate molasses in reversing hematological changes in rats exposed to Phenylhydrazine, through evaluating CBC, hemoglobin electrophoresis, G6PD, Iron, erythropoietin and histopathological examination of spleen.

Materials and Methods

Plant material

Pomegranates were obtained from an indigenous market at November 2017. The peels were peeled off and the seeds were squeezed to obtain the juice. Nine liters of pomegranate fresh juice were filtered to remove seeds and subjected to lyophilization Freez dryer (Model SB4, England Chemlab, and England) to give 770 gram of pomegranate molasses.[3, 4]

Experimental animals

The study was performed using forty male W/A rats 6-8 weeks old, weighing 250-275 g (Zagazig University, Zagazig, Egypt). The rats were housed in clear polypropylene cages and provided free access to purified water and standard rodent pellets. Constant animal housing conditions were applied constituting alternating 12 hours light and dark, a temperature of 22± 3°C, relative humidity of 50-60%, and adequate ventilation. The experimental design and animal handling procedures were as indicated by the guidelines of the Ethical Committee for Animal Handling- at Zagazig University (ZU-IACUC/2/F/26/2022).

Experimental protocols

After acclimatization, animals were randomly divided into four groups (ten each), the first group (control) received corn, maize and barley for two weeks. The second group received molasses pomegranate pure group (0.25 ml/kg body weight) orally for two weeks. The third group received Phenylhydrazine group (0.75 ml/ kg body weight); rats were given intraperitoneally for 3 days. The fourth group received molasses

pomegranate (0.25 ml/kg body weight, p.o.) + Penylhydrazine (0.75 ml/ kg body weight, i.p.) for 3 days with continued giving molasses pomegranate extract.
At the end of the experiment the rats were fasted overnight and sacrificed and blood samples were drawn from the orbital sinus of eyes; heparinized blood obtained for CBP and another blood sample was allowed to clot at room temperature for 20- 30 minutes, and then centrifuged at 3000 rpm for 15 minutes. The sera were kept at-20°C until used for analysis. The spleen was removed and fixed in 10% neutral buffered formalin for histopathological examinations.

Hematological parameters
In heparinized-blood, red blood cells count (RBCs), reticulocyte counts, packed cell volume (hematocrit, PCV), mean cell volume (MCV), red cell distribution width-coefficient of variation (RDW-CV), hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were measured using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy).[5]

Osmotic fragility Test
When subjected to hypotonic solutions, microcytic red blood cells are resistant to lysis.[6]
Red Blood Cell Survival: RBCs turnover rate (percent/day) = 100÷ RBCs survival (days).[7]

Hemoglobin Electrophoresis
The objective of Helena's Hemoglobin Electrophoresis Procedure, which employs cellulose acetate in an alkaline solution, is to identify aberrant hemoglobins both qualitatively and quantitatively.[8]

Erythropoietin and Iron level assay
A Sandwich ELISA technique was used to measure the amount of erythropoietin in the serum using EPO ELISA kits from MD Biosciences in St. Paul, Minnesota, USA. Using the Roche modular P800 (Roche, Milan, Italy), the Ferene technique was used to measure the serum iron content. [9, 10]

Glucose-6-Phosphate Dehydrogenase (G6PD)
Using the Colorimetric G6PD Assay Kit (Biomed Diagnostics, Inc.) in accordance with the manufacturer's instructions, G6PD activity in RBCs was assessed.[11]

Histopathological examination
Spleen samples that had been preserved in neutral buffered formalin at 10% were encased in blocks of paraffin. For histological analysis, 5 µm thick paraffin-embedded tissue slices were cut, dewaxed in xylene, hydrated with graded ethanol, and stained with hematoxylin and eosin (H&E) dyes. Light microscopy was used to analyze the slides.[12]

Statistical analysis
A software statistical program called SPS is used for social sciences for interactive or batch statistical analysis. International Business Machines (IBM) purchased SPSS Inc., which had been manufactured in 2009. The most recent (2015) versions are known as IBM SPSS Statistics. ANOVA is a series of statistical models and related processes (such as "variation" within and across groups) that are used to examine the variances in group means. Ronald Fisher, a statistician and evolutionary biologist, created the ANOVA. F-test: Any statistical test with an F-distribution, for the test statistics under the null hypothesis is known as an F-test. Post-hoc evaluation is a kind of scientific research. Post hoc analysis refers to studies that weren't planned ahead of time (from the Latin post hoc, "after this").[13]

Results and Discussion

Red blood cells count and blood indices parameters: There was a significant decrease (P< 0.0001) in red cell count level in PHZ group (2.5±1.026 mill/ cmm), when compared with control group (7.03± 0.845 mill/ cmm). However, there was a significant increase (P< 0.0001) in red blood cell count levels in molasses pomegranate group (7.009± 0.893 mill/ cmm), when compared with PHZ group (2.5± 1.026 mill/ cmm). In addition, there was a significant increase (P< 0.001) in red blood cell count level in Co-administered Phenylhydrazine group with molasses (3.69± 0.67 mill/cmm), when compared with PHZ group (2.5± 1.026 mill/ cmm), as shown in Table 1. Regarding, the blood indices, there was non-significant (P≥ 0.05) in mean corpuscular volume levels in co-administered Phenylhydrazine group (78.38± 9.79 Fl) with molasses, when compared with PHZ group (88.3± 17.45 Fl). Also, there was a significant increase (P< 0.0001) in mean corpuscular hemoglobin level in PHZ group (32.25± 5.005 Pg), when compared with control group (21.89± 1.521 Pg). However, non-significant change (P≥ 0.05) in mean corpuscular hemoglobin levels was observed in molasses pomegranate group (21.8± 1.429 Pg), when compared with control group (21.89± 1.521 Pg). In addition, a significant decrease (P< 0.0001) in mean corpuscular hemoglobin levels in molasses pomegranate group (21.8± 1.429 Pg) was observed, when compared with PHZ group (32.25± 5.005Pg), as shown in Table 1. Table 1, indicates that - there was a significant increase (P< 0.0001) in mean corpuscular hemoglobin concentration level in PHZ group (36.85± 2.67 g/dl), when compared with control group (32.11± 1.22 g/dl). However, there was non-significant (P≥ 0.05) in mean corpuscular hemoglobin concentration levels in molasses pomegranate group (31.78± 1.98 g/dl) when compared with control group (32.11± 1.22 g/dl). Also, there was a significant decrease (P< 0.0001) in mean corpuscular hemoglobin concentration levels in molasses pomegranate group (31.78± 1.98 g/dl), when compared with PHZ group (36.85± 2.67 g/dl). Concerning, red blood cell distribution width levels, Table (1) showed a significant increase (P< 0.0001) in red blood cell distribution width level in PHZ group (20.81± 2.166%) as compared with control group (15.86± 0.619%); however, there was non-significant change (P≥ 0.05) in red blood cell distribution width levels in molasses pomegranate group (15.83± 0.797%), when compared with control (15.86± 0.619%). Also, a significant decrease (P< 0.0001) in red blood cell distribution width levels in molasses pomegranate group (15.83± 0.797%) as compared with PHZ group (20.81± 2.166%). Also, a significant increase (P< 0.01) in red blood cell distribution width level in molasses pomegranate+ PHZ group (23.75± 0.996%), when compared with PHZ group (20.81± 2.166%).
The Osmotic Fragility (Initial hemolysis) levels, results (Table 2) revealed a significant increase (P< 0.0001) in osmotic fragility (initial hemolysis) level in PHZ group (0.88± 0.042%), when compared with control group (0.6± 0.082%) but, there was non-significant (P≥ 0.05) in osmotic fragility (initial hemolysis) levels in molasses pomegranate group (0.56± 0.07%) when compared with control (0.6± 0.082%).

Table 1: Red blood cells count and blood indices parameters

Groups	Red Blood Cells (RBC) (mill/cmm)	Mean Corpuscular Volume (MCV) (fl)	Mean Corpuscular Hemoglobin (MCH) (pg)	Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dl)	Red Blood Cell Distribution Width (RDW-CV) (%)
Control	7.03 ± 0.845	68.48 ± 5.452	21.89 ± 1.521	32.11 ± 1.22	15.86 ± 0.619
Molasses	7.009 ± 0.893	68.63 ± 5.094	21.8 ± 1.429	31.78 ± 1.98	15.83 ± 0.797
PHZ	2.5 ± 1.026	88.3 ± 17.45	32.25 ± 5.005	36.85 ± 2.67	20.81 ± 2.166
Molasses+PHZ	3.69 ± 0.67	78.38 ± 9.79	26.44 ± 2.711	35.59 ± 6.502	23.75 ± 0.996

Also, a significant decrease (P< 0.0001) in osmotic fragility (initial hemolysis) levels in molasses pomegranate (0.56± 0.07%) was observed when compared with PHZ group (0.88± 0.042%). Furthermore, there was a significant decrease (P< 0.0001) in osmotic fragility (initial hemolysis) levels in molasses pomegranate+ PHZ (G6) when compared with PHZ (G2): (0.88± 0.042). Effects on Osmotic Fragility (Complete hemolysis) levels: There was a significant increase (P< 0.0001) in osmotic fragility (complete hemolysis) level in PHZ group (0.55± 0.071%), when compared with control (0.29± 0.088%) but, there was non-significant change (P≥ 0.05) in osmotic fragility (complete hemolysis) levels in molasses pomegranate group (0.29± 0.074%), when compared with control (0.29±0.088%). Also, a significant decrease (P< 0.0001) in osmotic fragility (complete hemolysis) levels in molasses pomegranate group (0.29± 0.074%) was detected when compared with PHZ group (0.55± 0.071%). Moreover, there was a significant decrease (P< 0.01) in osmotic fragility (complete hemolysis) levels in molasses pomegranate+ PHZ group (0.45± 0.071%), when compared with PHZ group (0.55± 0.071%), as shown in Table (2). Effects on red blood cell survival levels: The results from Table (2) showed a significant decrease (P< 0.0001) in red blood cell survival level in PHZ group (15.06± 2.021 days), when compared with control (23.37± 0.538 days). Non-significant change (P≥ 0.05) in red blood cell survival levels was detected in molasses pomegranate group (22.73± 1.595 days) when compared with control (23.37± 0.538 days). In addition, a significant increase (P< 0.0001) in red blood cell survival levels in molasses pomegranate group was determined (22.73± 1.595 days) when compared with PHZ group (15.06± 2.021 days). Also, non-significant change (P≥ 0.05) in red blood cell survival levels in molasses pomegranate+ PHZ group (15.5± 0.956 days), when compared with PHZ group (15.06± 2.021 days). Effect on Reticulocytes: A significant increase (P< 0.0001) in reticulocytes level was detected in PHZ group (15.37± 3.347), when compared with control group (2.68± 0.539). However, there was non-significant change (P≥ 0.05) in reticulocytes levels in molasses pomegranate group (2.9±0.24), when compared with control (2.68± 0.539). There was a significant decrease (P< 0.0001) in reticulocytes levels in molasses pomegranate group (2.9± 0.24), when compared with PHZ group (15.37± 3.347). Also, a significant decrease (P< 0.001) in reticulocytes level in molasses pomegranate+ PHZ group (11.8± 2.276), when compared with PHZ group (15.37± 3.347) was determined, as shown in Table (2). Effects on Hemoglobin Concentration levels: A significant decrease (P< 0.0001) in hemoglobin concentration level in PHZ group (7.67± 2.14 g/dl) was detected when compared with control (15.29± 1.17 g/dl). While there was non-significant (P≥ 0.05) in hemoglobin concentration levels in molasses pomegranate (15.18±1.63 g/dl) when compared with control (15.29± 1.17 g/dl). Moreover, a significant increase (P< 0.0001) in hemoglobin concentration levels in molasses pomegranate group (15.18± 1.63 g/dl) was observed when compared with PHZ group (7.67± 2.14 g/dl). Also, a significant increase (P< 0.01) in hemoglobin concentration levels in molasses pomegranate+ PHZ group (9.66± 1.053 g/dl), when compared with PHZ group (7.67± 2.14 g/dl), as shown in Table (2). Effects on Hematocrit (PCV) value: There was a significant decrease (P< 0.0001) in hematocrit (PCV) level in PHZ group (20.71± 5.19%) when compared with control (47.67± 2.37%). On the other hand, non-significant change (P≥ 0.05) in hematocrit (PCV) values was detected in molasses pomegranate group (47.2± 2.91%) as compared with control (47.67± 2.37%). Furthermore, a significant increase (P< 0.0001) in hematocrit (PCV) levels in molasses pomegranate (47.2±2.91%) was observed as compared with PHZ group (20.71± 5.19%). Additionally, there was a significant increase (P< 0.001) in haematocrit (PCV) levels in molasses pomegranate+ PHZ group (28.63±7.74%) when compared with PHZ group (20.71± 5.19%), as shown in Table 2.

Effect on Hemoglobin Electrophoresis: There was non-significant (P≥ 0.05) in hemoglobin adult (Hb A) levels in PHZ group (96.91± 0.981 %), molasses pomegranate group (97.07± 1.146%), when compared with control (97.23± 0.618%). Moreover, non-significant change (P≥ 0.05) in hemoglobin adult (Hb A) levels in molasses pomegranate group (97.07± 1.146%) was detected when compared with PHZ group (96.91± 0.981 %). Non-significant change (P≥ 0.05) in hemoglobin adult (Hb A) levels in molasses pomegranate+ PHZ group (96.79± 0.936%) was observed, when compared with PHZ group (96.91± 0.981 %), as shown in Table 3. Effects on Normal Adult Hemoglobin (Hb A2) levels: Non-significant change (P≥ 0.05) in normal adult hemoglobin (Hb A2) levels was detected in PHZ group (2.59± 1.168 %), molasses pomegranate group (2.97± 1.136%), when compared with control (2.77± 0.618%). Also, there was non-significant (P≥ 0.05) in normal adult hemoglobin (Hb A2) levels in molasses pomegranate group (2.97± 1.136%), when compared with PHZ group (2.59± 1.168 %), as shown in Table (3). Effects on Hemoglobin Crystallization (Hb C) levels: A significant increase (P< 0.0001) in hemoglobin crystallization (Hb C) level was determined in PHZ group (0.5± 0.447 %), when compared with control (0±0 %). However, there was non-significant (P≥ 0.05) in hemoglobin crystallization (Hb C) levels in molasses pomegranate group, when compared with control (0±0 %). Also, a significant decrease (P < 0.0001) in hemoglobin crystallization (Hb C) levels was observed in molasses pomegranate (0± 0 %) group when compared with PHZ group (0.5± 0.447%). There was non-significant change (P≥ 0.05) in hemoglobin crystallization (Hb C) level in molasses pomegranate+ PHZ group (0.42± 0.394%), when compared with PHZ group (0.5±0.447%), however, as shown in Table 3.

Table 2: Red Blood Cells Parameters

Groups	Osmotic Fragility (Initial hemolysis) (%)	Osmotic Fragility (Complete hemolysis) (%)	Red Blood Cell Survival (days)	Reticulocytes (%)	Haemoglobin Concentration (g/dl)	Haematocrit (%)
Control	0.6 ± 0.082	0.29 ± 0.088	23.37 ± 0.538	2.68 ± 0.539	15.29 ± 1.17	47.67 ± 2.37
Molasses	0.56 ± 0.07	0.29 ± 0.074	22.73 ± 1.595	2.9 ± 0.24	15.18 ± 1.63	47.2 ± 2.91
PHZ	0.88 ± 0.042	0.55 ± 0.071	15.06 ± 2.021	15.37 ± 3.347	7.67 ± 2.14	20.71 ± 5.19
Molasses+PHZ	0.7 ± 0.105	0.45 ± 0.071	15.5 ± 0.956	11.8 ± 2.276	9.66 ± 1.053	28.63 ± 7.74

Table 3: Hemoglobin Electrophoresis Parameters

Groups	Hemoglobin Adult (Hb A) (%)	Normal Adult Hemoglobin (Hb A2) %	Hb C (%)
Control	97.23 ± 0.618	2.77 ± 0.618	0 ± 0
Molasses	97.07 ± 1.146	2.97 ± 1.136	0 ± 0
PHZ	96.91 ± 0.981	2.59 ± 1.168	0.5 ± 0.447
Molasses+PHZ	96.79 ± 0.936	3.05 ± 0.919	0.42 ± 0.394

Effect on Iron level: Serum iron level showed a significant increase (P< 0.0001) in PHZ group (408.67±51.48 ug/dl), when compared with control (121.8± 4.84 ug/dl). However, there was a non-significant (P≥ 0.05) in the serum iron levels in molasses pomegranate group (121.9± 7.01), when compared with control (121.8± 4.84 ug/dl). Also, a significant decrease (P< 0.0001) was detected in molasses pomegranate group (121.9± 7.01 ug/dl) when compared with PHZ group (408.67± 51.48 ug/dl). While a significant decrease (P< 0.0001) in the serum iron levels was observed in molasses pomegranate + PHZ group (255.4± 15.69 ug/dl), when compared with PHZ group (408.67± 51.48 ug/dl), as shown in Table (4). Effect on Erythropoietin level: Serum erythropoietin level revealed a significant increase (P < 0.0001) in the in PHZ group (45.09± 9.55 mIU/ml), when compared with control (5.74± 0.53 mIU/ml). On one hand, there was non-significant change (P≥ 0.05) in molasses pomegranate group (6.71± 1.44) when compared with control (5.74± 0.53 mIU/ ml). On the other hand, a significant decrease (P< 0.0001) in the serum erythropoietin level was observed in molasses pomegranate group (6.71± 1.44 mIU/ml) when compared with PHZ group (45.09±9.55 mIU/ml). In addition, there was significant decrease (P< 0.0001) in the serum erythropoietin levels in molasses pomegranate+ PHZ group (28.62± 4.09 mIU/ml) when compared with PHZ group (45.09± 9.55 mIU/ml), as shown in Table (4). Effect on Glucose-6-Phosphate Dehydrogenase: Table 4 shows a significant decrease (P< 0.0001) in the serum glucose-6-phosphate dehydrogenase level in PHZ group (2.54± 0.425 U/gm Hb), when compared with control (7.09± 0.586 U/gm Hb). While non-significant change (P≥ 0.05) was detected in molasses pomegranate group (7.76± 0.714 U/gm Hb), when compared with control (7.09± 0.586 U/gm Hb). Moreover, there was a significant increase (P< 0.0001) in molasses pomegranate group (7.76±0.714) when compared with PHZ group (2.54± 0.425 U/gm Hb). In molasses pomegranate+ PHZ group (3.31± 0.412 U/gm Hb), serum glucose-6-phosphate dehydrogenase level showed a significant increase (P< 0.0001) when compared with PHZ group (2.54± 0.425 U/gm Hb).

The results of this investigation revealed that administration of phenylhydrazine to rats induced decrease of Red Blood Cells, hematocrit, hemoglobin, serum iron, and copper causing anemia. These results are in agreement with those reported by,[31] who demonstrated that hematological data showed a significant decrease in hemoglobin concentration, RBCs count and HCT values following PHZ administration.

Development of anemia could be attributed to the toxicity induced by phenylhydrazine, inducing peroxidation of RBCs membrane lipids; decreased red blood cell survival happens because of the increased membrane fragility, reduced RBCs count, decreased hemoglobin production, and auto-oxidation of the drug and the interaction of oxygen radicals with membrane lipids.[32]

Pomegranate is high in natural antioxidants (anthocyanins, catechins, quercetin, gallotannins, ellagitannins, ellagic, ferulic, and gallic acid), which have promising antioxidant properties. Ellagitannins (punicalagin and its derivatives) are the most abundant polyphenols in the peel, and they are responsible for the peel's potent antioxidant properties.[30]

Pomegranate juice's ability to raise blood haemoglobin levels is supported by rising hemoglobin levels which is in line with the result of this study.[16]

The results of the reticulocytes and erythrocytes support previous report,[17] which revealed that greasy acids make up the lipids in reticulocytes and erythrocytes.

According to the findings of this study, levels of Red Blood Cells, Hemoglobin, Hematocrit, and Reticulocytes decrease noticeably and increase in comparison to phenylhydrazine group. This is consistent with the findings of.[19] In comparison to PHZ group, PHZ+ TQ group had high levels of RBCs, hemoglobin, hematocrit, and reticulocytes.

The study revealed that RBCs, Hemoglobin, Hematocrit, MCHC, MCV, and Osmotic Fragility are in accordance with previous report. [20] In the show discussion, it revealed that MCH is neutral with regard to.[20] In the display ponder, researchers found in RBCs, hematocrit, Hemoglobin, MCH, MCHC, EPO, is implied in.[3]

Pomegranate juice appears to be a potent anti-inflammatory and anti-thrombocytopenia among the older population. [21] It is encouraged to incorporate such supplements into the dietary needs of the elder population.

It has been shown that the antioxidant activity of pomegranate is present in both red and white peels, [23] and may be used as an excellent, affordable source of natural cancer prevention.[22]

The hemoglobin, RBC, Hematocrit, and Osmotic Fragility were in agreement with the reports of previous studies. [1, 24, 25, 26]

Regarding erythropoietin levels, [33] reported that renal cells produce erythropoietin hormone in response to renal hypoxia, which could be caused by anemia, which could account for the high levels of erythropoietin in the rats given PHZ alone or concurrently with pomegranate molasses.

Erythropoiesis is regulated by the glycoprotein hormone erythropoietin, which is increased in the serum of PHZ-infused organisms.[18] This demonstrates the compartmentalization of EPO synthesis inside the rodent and the ability of the rodent liver to produce EPO under high pressure.[15]

Histopathology of Spleen

Due to deterioration inside the red and white pulp with edematous spaces, the PHZ-exposed group has a greater prevalence of macrophages harboring cellular debris. Additionally, PHZ offered an enlarged picture of the final field that showed macrophages, vacuolization, and necrotic areas present inside the white pulp. The last field inside the red pulp is similarly enlarged, and this image reveals a considerable number of necrotic cells, hemorrhage, and occasional apoptotic cells. The usual red and white pulps as well as increased red phenol pigment deposition were present in rats administered pure pomegranate molasses. When PHZ-exposed mice were treated with molasses pomegranate (Molasses Pomegranate + PHZ), the splenic parenchyma in the red and white pulps had a decreased degree of recovery, the presence of apoptotic cells, coupled with bleeding and vacuolization – (Figure 1).

In the present investigation, researchers found that degeneration inside the white pulp and red pulp with edematous gaps was associated with a greater incidence of macrophages harboring cellular debris in the PHZ-exposed group. Additionally, PHZ featured an enlarged section of the final field that demonstrated the presence of macrophages clearly within the white pulp, as well as vacuolization and necrotic regions. A part of the final field within the red pulp that has been enlarged, reveals a significant number of necrotic cells, hemorrhage, and sporadic apoptotic cells. Findings of the current research are consistent with.[27]

In the present investigation, researchers found that molasses pomegranate treatment (Molasses Pomegranate + PHZ) resulted in decreased recovery of the splenic parenchyma in the red and white pulps, as well as the presence of dead cells, hemorrhage, and vacuolization. Study results are in line with.[27]

Table 4: Iron, Erythropoietin and Glucose-6-Phosphate Dehydrogenase parameters

Groups	Iron (ug/dl)	Erythropoietin (EPO) (mIU/ml)	Glucose-6-Phosphate Dehydrogenase (G6PD) (U/gm Hb)
Control	121.8 ± 4.84	5.74 ± 0.53	7.09 ± 0.586
Molasses	121.9 ± 7.01	6.71 ± 1.44	7.76 ± 0.714
PHZ	408.7 ± 51.48	45.09 ± 9.55	2.54 ± 0.425

Molasses+PHZ	255.4 ± 15.69	28.62 ± 4.09	3.31 ± 0.412

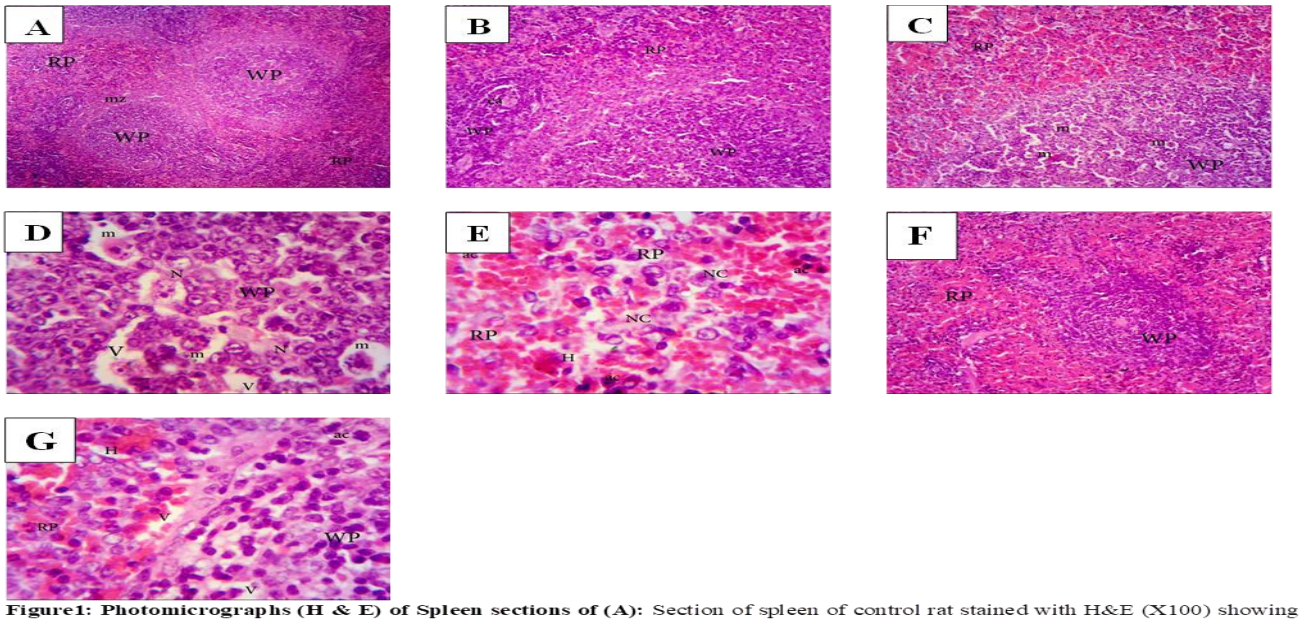

Figure1: Photomicrographs (H & E) of Spleen sections of (A): Section of spleen of control rat stained with H&E (X100) showing normal histological structure with the lymphatic nodules of white pulp (WP) surrounded with a mantle zone and the red pulp (RP). **(B):** A magnified portion of the last field (X200) showing the central arteriole (ca) inside the lymphatic nodules of white pulp (WP) and the cell cords of the red pulp (RP). **(C):** Section of spleen of a rat exposed to PHZ (1 mg) stained with H &E (X200) showing increased incidence of macrophages (m) containing cellular debris due to degeneration inside the white pulp (WP) and red pulp (RP) with edematous spaces (asterisk). **(D):** A magnified portion of the last field (X1000) showing the incidence of macrophages (m) clearly inside the WP together with vacuolization (V) and necrotic areas (N). **(E):** Another magnified portion of the last field (X1000) inside the red pulp (RP) showing a large number of necrotic cells (NC), hemorrhage (H) and scattered apoptotic cells (ac). **(F):** Section of spleen of pure pomegranate molasses- administered rat stained with H&E (X200) showing normal red and white pulps (RP and WP respectively) with increased deposition of red phenol pigments (asterisk. **(G):** Section of spleen of PHZ-exposed rat treated with molasses pomegranate (Molasses + PHZ) stained with H&E (X1000) showing a less degree of recovery in splenic parenchyma in red and white pulps (RP and WP respectively) and the presence of apoptotic cells (ac) together with hemorrhage (H) and vacuolization (V).

Dead RBCs is stored in the spleen, which is also where hemoglobin is degraded. Due to the faster hemoglobin breakdown in hemolytic anemia, there is an increase in iron deposition in the spleen. [27] provided evidence that PHZ treatment groups' splenic fibrosis and necrosis were caused by this. The pomegranate molasses treatment considerably corrected this cytoarchitecture disturbance. Fruit juice performed significantly better in this aspect because, in addition to reducing fibrosis, it also moderately restored cellularity, preventing toxicant-induced cell loss. It is conceivable to conclude that *O. elatior* fruit juice helps to reverse the toxic effects of PHZ on the spleen based on the biochemical and histological data.

The researchers found that rats given pure molasses-pomegranate had normal red and white pulps with higher red phenol pigment deposition in the current investigation. This research results are in agreement with.[28, 29]

The immunostimulatory effects of pomegranate may be the cause of the rise in globulin levels.[28] Pomegranate has been reported to significantly stimulate both humoral and cell-mediated immune responses in rabbits. Additionally, pomegranate increases immunoglobulin synthesis in mice spleen cells and may enhance B cell activity *in vivo*.[29]

The researchers found that rats given molasses-pomegranate pure had normal red and white pulps with higher red phenol pigment deposition in the current investigation. This study results agree with those in.[14]

Furthermore, the proinflammatory cytokines IL-1, TNF-, iNOS, and IFN were connected to the modulatory action of pomegranate peel extract (PPE) on splenic damage and oxidative stress. The results of the study on PPE's effects on IL-1, TNF-, iNOS, and IFN mRNA expression levels were remarkable.[14] The findings demonstrated that the untreated infected group of rats had greater proinflammatory cytokine mRNA levels than the controls. The PPE-treated group of infected rats, however, exhibited noticeably reduced mRNA levels in comparison to the untreated animals.

Conclusion

It could be concluded that pomegranate molasses, based upon hematological parameters, as well as the spleen examinations, ameliorated the deleterious effect of PHZ on heamatological parameters. Moreover, it improved the hematological parameters and had anti-inflammatory effect on spleen. These strong hematinic and anti-anemic benefits could be attributed to their antioxidant activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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