Pomegranate (Molasses, White Peel, Red Peel) effect in reducing Phenylhydrazine-induced Spleen injury and hemolytic anemia incidence

Nabil Abbas Soliman  
Zagazig University

Sherif Wajih Mansour  
Zagazig University

Mohammed Ahmed Ammar (mhsoon5593n@gmail.com)  
Zagazig University

Mohamed Ahmed Mohamed Ammar  
Zagazig University

Research Article

Keywords: molasses pomegranate, white peel pomegranate, red peel pomegranate, hematology, spleen

Posted Date: January 11th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2319307/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

In this study, the effects of pomegranate molasses, white peel extract, and red peel extract on male rats with phenylhydrazine-induced anaemia are discussed. Reduced body weight, haemoglobin concentration, hematocrit, red cell count, and red blood cell survival were the results of phenylhydrazine-induced anaemia. Adult haemoglobin type A, MPV, basophils, PDW, normal adult haemoglobin type A2, eosinophils, PDW, and PCT. Initial hemolysis due to fragility, complete hemolysis due to osmotic fragility, Adult haemoglobin type A, normal adult hemoglobin, MCHC, RDW-CV, PCT, and PDW, as well as eosinophils. In the histopathology analysis of the spleen, the PHZ exposed group had a higher incidence of macrophages carrying cellular debris as a result of degeneration inside the white pulp and red pulp with edematous gaps. Additionally, PHZ provided a magnified view of the final field that demonstrated the presence of macrophages inside the white pulp along with vacuolization and necrotic regions. A magnified view of the last field inside the red pulp is also shown, revealing a significant amount of necrotic cells, bleeding, and sporadic apoptotic cells. There was an increase Body Weight, G6PD, Hemoglobin Concentration, Hematocrit, Red Cell Count, Red Blood Cell Survival, PDW, Normal Adult Hemoglobin Hb A2, MPV, MCHC, Basophils, Hemoglobin Adult Hb A, RDW, PCT, Eosinophils. The splenic parenchyma in the red and white pulps of PHZ-exposed rats receiving molasses pomegranate treatment showed a lesser degree of recovery and the presence of apoptotic cells along with bleeding and vacuolization. Strong antioxidants in molasses, white peel, and red peel of pomegranates preserve haematological and spleen tissue.

Highlights

- Improvement of hematological parameters and spleen tissue by taking extract of pomegranate molasses and peels.
- After phenylhydrazine delivery, hematological marker significantly changed.
- However, pretreatment with pomegranate and peels significantly decreased this toxicity via altering the spleen tissue and hematological biomarkers. (The extract of molasses pomegranate, white peel and red peel pomegranate showed strong hematinic and anti-anemic benefits due to their antioxidant activity).

Introduction

Phenylhydrazine causes hemolytic anaemia by causing red blood cells to be destroyed by oxidative stress and a variety of other cellular alterations. The pathophysiology of hemolytic anaemia, the impact of anaemia on other physiological systems, and the development of related disorders can all be studied using the PHZ-induced toxic anaemia as a model (1).

In Turkish cuisine, pomegranate molasses (PM) is used as a condiment and is thought to have significant effects on atherosclerosis, cholesterol levels, and cancer prevention due to the pomegranate fruit's inherent antioxidant potential. This information was gathered by (2). They came to the conclusion that a variety of factors, including the cultivar, the climatic circumstances during fruit ripening and maturity, and the part of the fruit, affect the antioxidant activity of pomegranate molasses.

The possible antimalarial, antioxidant, and anti-inflammatory properties of pomegranates were reviewed by (3). Additionally, when compared to the untreated group, pomegranate peel extracts dramatically decreased the parasitemia and spleen index of the treated mice. Additionally, the results were corroborated by the spleen histology score, which revealed greater improvement in the treated mice compared to the control mice.
According to research by (4), plant extracts could be a significant substitute for synthetic antioxidants in the oil sector. Pomegranate peel is regarded as an affordable, plentiful, and enhanced source of functional ingredients as well as a sustainable source for the extraction of polyphenolic and flavonoid chemicals, which have an exceptional ability as antioxidants.

**Materials And Methods**

**Plant material:**

Pomegranates were obtained from an indigenous market and peeled off then the seeds were squeezed to obtain the juice. 9 liters of pomegranate fresh juice were filtered to remove seeds and then subjected to lyophilization Freez dryer (Model SB4, England Chemlab, England) to give 770 gram of pomegranate molasses.

**White peel pomegranate:** pomegranate white peel (fresh mesocarp) were cut into small piece, air dried for few days to give 235gm powder. The powdered drug was extracted with 80% aqueous ethyl alc (4×8L), the solvent was removed under reduced pressure to gives 155gm viscous residue and 27gm of viscous residue were added to 200ml distilled water.

**Red peel pomegranate:** pomegranate red peel (leathery mesocarp) were cut into small piece, air dried for few days to give 202gm powder. The powdered drug was extracted with 80% aqueous ethyl alc (4×8L), the solvent was removed under reduced pressure to gives 155gm viscous residue then 17gm of viscous residue were added to 200ml distilled water.

**Biological Study**

**Experimental animals**

Rats used in this study were male Wistar 6-8 weeks old rats weighing 250-275 g (Zagazig University, Zagazig, Egypt). Each 4 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).

**Study design**

Animals were randomly divided into 8 experimental groups, each of 80 male rats. **Group 1 (C):** control rats that received regular tap water and food pellets for 2 weeks. **Group 2 (C-M):** control rats received molasses extract (40 mg/kg/day) for 2 weeks. **Group 3 (C-WPP):** control rats received WPP extract (dose) for 2 weeks. **Group 4 (C-RPP):** control rats received (dose) for 2 weeks. **Group 5 (PHZ):** rats received i.p. injection of PHZ in saline in a dose 50 mg/kg/day at the last three days of the 2 weeks of the study. **Group 6 (PHZ-M):** rats received molasses for 2 weeks and PHZ at the last three days of the 2 weeks. **Group 7 (PHZ-WPP):** rats received WPP for 2 weeks and PHZ at the last three days of the 2 weeks. **Group 8 (PHZ-RPP):** rats received RPP for 2 weeks and PHZ at the last three days of the 2 weeks.

**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), total leukocyte count, relative differential leukocytes count including neutrophils and lymphocytes and platelet count were measured using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy) (5).

Osmatic fragility Test

Microcytic red blood cells are resistant to lysis when exposed to hypotonic solutions (6).

Blood Red Cell Survival

Estimation of red cell survival from the reticulocyte count - In order to maintain a constant RBC mass, the bone marrow must produce as many new RBCs (reticulocytes) per day as are being destroyed (7). At a mean RBC survival of 100 days, 1 percent of the RBC mass is destroyed and replaced each day. This is the RBC turnover rate, the reciprocal of RBC survival:

RBC turnover rate (percent/day) = 100 ÷ RBC survival (days)

Hemoglobin Electrophoresis procedure

Helena's Hemoglobin Electrophoresis Procedure, using cellulose acetate in alkaline buffer, is intended for the qualitative and quantitative determination of abnormal hemoglobins (8).

Erythropoietin and Iron level assay

Serum level of erythropoietin was assayed using Sandwich ELISA technique using EPO ELISA kits (M D Biosciences, St Paul, MN, USA). The serum iron concentration was detected by the Ferene method using Roche modular P800 (Roche, Milan, Italy) (9; 10).

Glucose-6-Phosphate Dehydrogenase (G6PD) analysis

G6PD activity in RBC's was measured following the manufacturer’s instructions using Colorimetric G6PD Assay Kit (Biomed Diagnostics, Inc) (11).

Histopathological examination

Spleen specimens that were fixed in 10% neutral buffered formalin were embedded in paraffin blocks. Paraffin-embedded tissue sections (5 µm thick) were cut, dewaxed in xylene, hydrated using graded ethanol, and stained with hematoxylin and eosin (H&E) dyes for histopathological examination. The slides were examined by light microscopy (12).

Drugs and chemicals

The following drugs and chemicals were used in this study: ketamine (Sigma pharmaceutical industries, Menoufia, Egypt) and xylazine (Sigma-Aldrich, St. Louis, MO, USA) for rat’s anesthesia (13).

Statistical analysis
Values are expressed as mean ± standard error of the mean. Statistical comparisons were carried out using one-way ANOVA, followed by Tukey’s Post hoc test using Prism 5® software (Graphpad, CA, USA). Probability levels less than 0.05 were considered statistically significant (14).

Results

Phenylhydrazine induced anemia resulted in: There was decreasing Body Weight, Hemoglobin Concentration, Hematocrit, Red Cell Count, Red Blood Cell Survival, Hemoglobin Adult Hb A, MCH, Basophils, PDW, Normal Adult Hemoglobin Hb A2, MPV, Eosinophils, PDW, PCT. Accompanied by increasing Iron, Erythropoietin, Hb C, MCV, MCH, Total Leucocytic Count, Platelets, Reticulocytes, Neutrophil, Lymphocytes, Monocytes, Osmotic Fragility: Initial hemolysis, Osmotic Fragility: Complete hemolysis, Hemoglobin Adult Hb A, MCHC, Normal Adult hemoglobin, MPV, RDW-CV, PCT, PDW, Eosinophils. In addition, Coombs Test Direct and Coombs Test Indirect are negative in all groups. Finally, Fetal Hemoglobin (Hb F) and Hb S: as a result, all the groups are 0. As Showing in Tables (1,2,3,4,5,6) and Figures (1,2,3,4,5,6).

Regarding the histopathology study of Spleen:

A higher incidence of macrophages harbouring cellular debris is seen in the PHZ-exposed group as a result of degeneration inside the red and white pulp with edematous gaps. Additionally, PHZ provided a magnified view of the final field that demonstrated the presence of macrophages inside the white pulp along with vacuolization and necrotic regions. A magnified view of the last field inside the red pulp is also shown, revealing a significant amount of necrotic cells, bleeding, and sporadic apoptotic cells. Rats given red peel pomegranate pure showed typical red and white pulps as well as a central arteriole with enhanced red phenol pigment deposition. Rats receiving only white peel pomegranate showed normal red and white pulps as well as a central arteriole with no histological alterations. Rats given pure pomegranate syrup showed typical red and white pulps as well as elevated red phenol pigment deposition. Red Peel Pomegranate + PHZ showed strong restored splenic parenchyma with good cellularity in the red and white pulps in the rat model of PHZ exposure. White Peel Pomegranate + PHZ showed largely restored splenic parenchyma in red and white pulps despite the presence of damaged and apoptotic cells in the rat model of PHZ exposure. The splenic parenchyma in the red and white pulps of PHZ-exposed rats receiving molasses pomegranate treatment (Molasses Pomegranate + PHZ) showed a lesser degree of recovery and the presence of apoptotic cells along with bleeding and vacuolization. As seen in Figures (7).

Discussion

In the current study, we found that Phenylhydrazine initiated frailty brought about in expanding Hemoglobin C. Our come about to concur with (15). Hemoglobin C illness is the result of a change within According to the results of the
current investigation, phenylhydrazine started the frailty that expanded haemoglobin C. Our development is in line with (15). A modification to the beta-globin causes haemoglobin C disease. Nearly 2% to 3% of African-Americans and West Africans have haemoglobin C.

In the show debate, we discovered that molasses, white peel, and red peel have improved Osmotic fragility. Ours develops through mutual understanding with (16, 17). The osmotic delicacy measurement used in this study by (17) revealed that Lantana camara extract appears to alter the film's acuity at NaCl concentrations close to physiologic levels. Similar to this, the morphological analysis of blood smears revealed that whole blood treated with Lantana camara extract of Lantana camera capable of connection with film components may influence the movement of particles into the erythrocyte layer or the adjustment of the osmotic pressure. It was explained that substances found in Lantana camara alter how protein C functions (16).

We discovered that Molasses Pomegranate, white peel, and red peel are expanded weight pick up in the display contemplate. Ours developed in cooperation with (18). (18) stated that the use of pomegranate peels should be encouraged during the manufacturing of natural products on the off chance that they appear to have the ability to improve human health.

We discovered in the display study that Molasses Pomegranate + PHZ, White Peel Pomegranate + PHZ, and Red Peel Pomegranate + PHZ contribute to the expansion of Hemoglobin C. Our results are consistent with (19, 20). According to research by (20), haemoglobin may contain a significant amount of press. The majority of the body's pressure comes from nutritional sources, and low pressure levels inside the body prevent the blood from successfully delivering oxygen.

Increasing haemoglobin level supports the role of pomegranate juice in raising blood haemoglobin levels. We discovered in the experiment that phenylhydrazine-induced frailty resulted in declining red blood cell survival. Our results are in agreement with (21).

These sensations can be used to translate the changes in osmotic delicacy that certain uncommon types of erythrocytes undergo after 24 hours of hatching (22). Our findings on reticulocytes and erythrocytes are consistent with those of (23), who discovered that the lipids in reticulocytes and erythrocytes are composed of greasy acids. The level in reticulocytes is significantly higher.

The rats who had received PHZ injections had their reticulocytes checked. A stamped leukocytosis with high amounts of lymphocytes, monocytes, and erythroid cells, in addition to alterations in erythroid cell number (24). The meaning of erythrocytes can be determined in the context of these views (22). Our findings on reticulocytes and erythrocytes are consistent with those of (23), who discovered that the lipids in reticulocytes and erythrocytes are composed of greasy acids. The level in reticulocytes is significantly higher.

The rats who had received PHZ injections had their reticulocytes checked. A stamped leukocytosis with high amounts of lymphocytes, monocytes, and erythroid cells, in addition to alterations in erythroid cell number (24). The glycoprotein hormone erythropoietin, which is elevated in the serum of PHZ-infused organisms, regulates erythropoiesis (25).

According to our results of red blood, haemoglobin, hematocrit, MCV, MCHC, erythropoietin, kidney, and liver, (13) found that red blood count, haemoglobin, and hematocrit levels in phenylhydrazine collection in 3 days were all reduced in comparison to control were accurate.
This shows that there is compartmentalization of EPO production inside the rodent, and that under conditions of high pressure, the rodent liver is also capable of producing EPO (13).

We discovered reticulocytes, bilirubin, LDH, and spread of fringe blood. According to G6PD and understanding with (26), reticulocytes, LDH, haptoglobin, and peripheral blood spread increase. insufficient G6PD (diminished). In agreement with (27); we discovered that molasses-pomegranate, white peel, and red peel are pharmacological medicines.

Due to the presence of a variety of bioactive chemicals, as shown in this audit, pomegranates are discovered to be connected to various pharmacological activities.

We found that RBC, Hb, PCV, MCHC, WBC, MCV, MCH, Iron, and this is often in understanding with (28) who detailed that, PHZ gather appeared a noteworthy diminish in RBC, Hb, PCV, MCHC and increment in WBC, MCV, MCH, Press, add up to iron-binding capacity (TIBC) compared to control bunch.

In line with our result in RBC, Hb, Hct, reticulocytes levels are understanding with (29) who expressed come about that, phenylhydrazine bunch: levels of RBC, Hb, Hct, Grass diminish noteworthy and increment reticulocytes levels compared to control bunch. PHZ + TQ bunch: levels of RBC, Hb, Hct, Grass, reticulocytes expanded levels compared to PHZ bunch.

Besides, our result in body weight, RBC, Hb, PCV, WBC, MCV, MCH, MCHC, Platelets, lymphocytes is in understanding with (30) who comes about that, phenylhydrazine bunch in body weight, RBC, Hb, PCV was diminished levels. But, WBC, MCV, MCH, MCHC, Platelets, lymphocytes were expanded levels compared to the control bunch. Iron deficient + MOE (tall dosage) bunch in body weight, WBC, RBC, Hb, PCV, platelets were expanded levels. But, MCV, MCH, MCHC, lymphocyte were diminished levels compared to the phenylhydrazine bunch. (30) explored that, the impact of ethanol extricate of Moringa oleifera takes off on hematology and serum lipid profile in PHZ-induced iron deficiency in Wistar rats. Moringa oleifera clears out have been detailed to have antitumor and anticancer action (31).

Within the display think about, we found in RBC, Hb, hematocrit, MCHC, MCV, WBC, Osmotic fragility are in agreement with (32). In the show ponder, we found in MCH, PLT is indifferent with (32).

In the display ponder, we found in RBCs, PCV, Hb, MCH, MCHC, Eosinophils, lymphocytes, EPO, total bilirubin, indirect bilirubin is in understanding with (33). In the display consider we found in WBCs, platelet, press, neutrophils, monocytes are different with (33).

Our result in Hemoglobin, RBC, MCHC, osmotic resistance of red blood cells, platelets, MCV upheld by (34) who concluded that the watery extricate of roots of Cocos nucifera was productive against frailty in a dose-dependent way. It invigorated erythropoiesis or maybe particularly and favors in iron deficiency recompense early stage, a discharge of youthful red blood cells within the circulation system.

Ours comes about for RBC, Hg, HCT, MCHC, RDW, MPV, PCT, WBC, and PDW are in assention with (35). Our result respect MCV, MCH, PLT contradiction with (35).

Bolstered by (35) who talked about that, pomegranate juice (PJ) utilization of 500 ml/day for two weeks expanded the RBC check, hemoglobin concentration, and hematocrit in solid people. Other parameters concerning total blood number, metabolic wellbeing, or irritation were not modified in this cohort of people. Subsequently, PJ admissions for a brief period may result in expanded erythropoiesis or avoidance of RBC debasement without any critical changes in components related in factors associated with metabolic health and inflammation in healthy individuals.
In the show consider, we found in platelets are advancement in pomegranate molasses, white peel pomegranate, and red peel pomegranate. Ours comes about is in understanding with (36). (36) concluded that characteristic pomegranate juice appears to be a powerful anti-inflammatory, anti-muscle harm, and anti-thrombocytopenia treatment among the elderly populace. In this manner, it’s exhorted to include such supplementation within the behavioral sustenance of the elderly population.

Within the show think about, we found in masses pomegranate; white peel pomegranate, and red peel are great cancer prevention agents. Our comes about are in assention with (37, 38).

Both red peels and white peels had high antioxidant exercises and can be considered as a great cheap source of normal cancer prevention agents. (37) demonstrated the most noteworthy antioxidant action of pomegranate peel water extricate (38). The action of these squanders might not depend on the substance of phenols but depends on the quality and chemical structure of these phenols. (38) considered that tactile assessment of pomegranate beverage indicated that the concentration of 1% of red peel refreshment was the foremost acknowledged in all traits having scores than white peel. It was cleared that the degree to which the panelists acknowledged the pomegranate peel refreshment, could be a great pointer of the plausibility of applying the generation of pomegranate red peel beverage.

Within the show ponder we found in Hb, RBC, PCV, Osmotic fragility, Neutrophil, Lymphocyte, Eosinophil, Basophil are assention with (1, 39, 40, 41). In the show ponder, we found in WBC, Monocytes are in contradiction with (1, 39, 40, 41). (41) assessed the impacts of Citrullus lanatus juice extricates on the hemolytic frailty initiated by PHZ in male Wistar rats. Iron deficiency could be a genuine issue of tall financial esteem in men and creatures (40). Subsequently, (41) examined that, a successful specialist oversees the iron-deficient condition in a cost-effective way. The Citrullus lanatus juice extricates successfully move forward the Hb, RBC, and PCV levels compared to the negative control bunch in an expanding dose-dependent way.

**Histopatolgy of Spleen**

In the current study, we discovered that the PHZ-exposed group had a higher incidence of macrophages containing cellular debris as a result of degeneration inside the white pulp and red pulp with edematous spaces. In addition, PHZ had a magnified portion of the last field showing the presence of macrophages clearly inside the white pulp, as well as vacuolization and necrotic areas. A magnified portion of the last field within the red pulp also shows a large number of necrotic cells, haemorrhage, and scattered apoptotic cells. Our findings are consistent with (42).

We discovered that PHZ-exposed rats treated with red peel pomegranate (Red Peel Pomegranate + PHZ) had good recovered splenic parenchyma with good cellularity in red and white pulps. Our findings are consistent with (42). We discovered that PHZ-exposed rats treated with white peel pomegranate (White Peel Pomegranate + PHZ) had partially-recovered splenic parenchyma in red and white pulps despite the presence of damaged and apoptotic cells. Our findings are consistent with (42).

In the current study, we discovered that PHZ-exposed rats treated with molasses pomegranate (Molasses Pomegranate + PHZ) had less recovery in splenic parenchyma in red and white pulps, as well as the presence of apoptotic cells, haemorrhage, and vacuolization. Our findings are consistent with (42).

The spleen is a repository for dead RBC, and it is also where Hb is broken down. Hemolytic anaemia causes an increase in iron deposition in the spleen due to the accelerated breakdown of haemoglobin. (42) demonstrated that the cause of splenic fibrosis and necrosis in PHZ-treated groups. The administration of the test drug significantly
reversed this disruption in the cytoarchitecture. Fruit juice was comparatively better in this regard because, in addition to attenuating fibrosis, it restored cellularity to a moderate level, thereby inhibiting toxicant-induced cell depletion. Based on the biochemical and histopathological findings, it is possible to conclude that *O. elatior* fruit juice aids in the reversal of the toxic effects of PHZ on the spleen.

The red peel pomegranate pure - administered rat showed normal red and white pulps and central arteriole with increased deposition of red phenol pigments in the current study. Our findings are consistent with (43, 44).

The white peel pomegranate pure - administered rat showed normal red and white pulps and central arteriole with no histopathological changes in the current study. Our findings are consistent with (43, 44).

In the current study, we discovered that molasses pomegranate pure - administered rats had normal red and white pulps with increased red phenol pigment deposition. Our findings are consistent with (43, 44).

The increase in globulin levels may be due to the immunostimulatory effect of pomegranate (43). Pomegranate was found to have significant immunostimulatory activity in rabbits, stimulating both humoral and cell-mediated immune responses. Pomegranate also promotes the production of immunoglobulins in mouse spleen cells and may improve the function of B cells *in vivo* (44).

The red peel pomegranate pure - administered rat showed normal red and white pulps and central arteriole with increased deposition of red phenol pigments in the current study. Our findings are consistent with (3).

The white peel pomegranate pure - administered rat showed normal red and white pulps and central arteriole with no histopathological changes in the current study. Our findings are consistent with (3).

In the current study, we discovered that molasses pomegranate pure - administered rats had normal red and white pulps with increased red phenol pigment deposition. Our findings are consistent with those of (3).

Furthermore, the modulatory effect of pomegranate peel extract (PPE) on splenic injury and oxidative stress was linked to proinflammatory cytokines IL-1, TNF-α, iNOS, and IFN. The effects of PPE on the expression levels of IL-1, TNF-α, iNOS, and IFN mRNA were studied, and the results were astounding (3). The results showed that the mRNA levels of proinflammatory cytokines were higher in the untreated infected group of mice compared to the controls. However, when compared to the untreated mice, the PPE-treated group of infected mice had significantly lower mRNA levels.

The red peel pomegranate pure - administered rat showed normal red and white pulps and central arteriole with increased deposition of red phenol pigments in the current study. Our findings are consistent with those of (45).

In the current study, we discovered that molasses pomegranate pure - administered rats had normal red and white pulps with increased red phenol pigment deposition. Our findings are consistent with those of (45).

The red colour of pomegranate juice has been attributed to anthocyanins, such as cyanidin, pelargonidin glycosides, and delphinidin, which have potent antioxidant activity, according to (45).

**Conclusion**

Strong antioxidants in molasses, white peel, and red peel of pomegranates preserve haematological and spleen tissue.
Declarations

Conflict of Interest

The authors have declared that no conflict of interests exist.

DECLARATION OF COMPETING

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

FUNDING SOURCES

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHORS CONTRIBUTION

MA performed the experiments, RA and NH prepared the manuscript. NS and SM revised the manuscript. All authors discussed the results and commented on the manuscript.

ETHICS APPROVAL STATEMENT

The animal study was reviewed and approved by the experimental design and animal handling procedures as indicated by the guidelines of the Ethical Committee for Animal Handling at Zagazig University (ZU-IACUC/2/F/26/2022).

ACKNOWLEDGMENT

We acknowledge initial support from Professor Abeer EL-Bayoumy from Department of physiology, Faculty of Medicine, Zagazig University.

References


Tables

Table 1: body weight and Red Blood Cells parameters:
Table (2): Red Blood Cells Parameters:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Osmotic Fragility (Initial hemolysis) (%)</th>
<th>Osmotic Fragility (Complete hemolysis) (%)</th>
<th>Red Blood Cell Survival (days)</th>
<th>Reticulocytes (%)</th>
<th>Haemoglobin Concentration (g/dl)</th>
<th>Haematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.6±0.082</td>
<td>0.29±0.088</td>
<td>23.37±0.538</td>
<td>2.68±0.539</td>
<td>15.29±1.17</td>
<td>47.67±2.37</td>
</tr>
<tr>
<td>PHZ</td>
<td>0.88±0.042</td>
<td>0.55±0.071</td>
<td>15.06±2.021</td>
<td>15.37±3.347</td>
<td>7.67±2.14</td>
<td>20.71±5.19</td>
</tr>
<tr>
<td>Molasses</td>
<td>0.56±0.07</td>
<td>0.29±0.074</td>
<td>22.73±1.595</td>
<td>2.9±0.24</td>
<td>15.18±1.63</td>
<td>47.2±2.91</td>
</tr>
<tr>
<td>White Peel</td>
<td>0.61±0.087</td>
<td>0.27±0.067</td>
<td>22.76±1.631</td>
<td>2.44±0.744</td>
<td>15.36±0.94</td>
<td>49.73±4.87</td>
</tr>
<tr>
<td>Red Peel</td>
<td>0.57±0.067</td>
<td>0.31±0.099</td>
<td>23.29±3.1</td>
<td>2.74±0.686</td>
<td>15.66±1.38</td>
<td>51.57±6.28</td>
</tr>
<tr>
<td>Molasses+PHZ</td>
<td>0.7±0.105</td>
<td>0.45±0.071</td>
<td>15.5±0.956</td>
<td>11.8±2.276</td>
<td>9.66±1.053</td>
<td>28.63±7.74</td>
</tr>
<tr>
<td>White Peel+PHZ</td>
<td>0.66±0.084</td>
<td>0.38±0.091</td>
<td>16.02±0.23</td>
<td>9.35±1.338</td>
<td>11.24±1.464</td>
<td>30.71±5.64</td>
</tr>
<tr>
<td>Red Peel+PHZ</td>
<td>0.56±0.084</td>
<td>0.32±0.092</td>
<td>18.57±0.495</td>
<td>4.91±1.502</td>
<td>14.05±1.85</td>
<td>39.16±6.21</td>
</tr>
</tbody>
</table>

Table (3): White Blood Cells and differential Parameters:
### Table (4): Platelets parameters:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Platelets (×10^3/cm)</th>
<th>Mean Platelet Volume (MPV) (fl)</th>
<th>Platelet Distribution Width (PDW) (%)</th>
<th>Plateletcrit (PCT) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>554.7±121.2</td>
<td>10.65±0.45</td>
<td>16.6±1.66</td>
<td>0.564±0.117</td>
</tr>
<tr>
<td>PHZ</td>
<td>793±73.11</td>
<td>10.79±0.6</td>
<td>14.78±1.34</td>
<td>0.495±0.152</td>
</tr>
<tr>
<td>Molasses</td>
<td>499.1±151.2</td>
<td>9.94±0.62</td>
<td>15.73±1.94</td>
<td>0.527±0.11</td>
</tr>
<tr>
<td>White Peel</td>
<td>481.5±102</td>
<td>10.67±0.51</td>
<td>15.47±2.34</td>
<td>0.537±0.073</td>
</tr>
<tr>
<td>Red Peel</td>
<td>467.8±106.6</td>
<td>10.68±1.19</td>
<td>15.52±1.16</td>
<td>0.581±0.039</td>
</tr>
<tr>
<td>Molasses+PHZ</td>
<td>747.7±41.74</td>
<td>11.46±0.657</td>
<td>13.2±0.573</td>
<td>0.419±0.024</td>
</tr>
<tr>
<td>White Peel+PHZ</td>
<td>720.1±35.14</td>
<td>10.7±0.707</td>
<td>14.93±1.171</td>
<td>0.463±0.115</td>
</tr>
<tr>
<td>Red Peel+PHZ</td>
<td>583.3±89.18</td>
<td>11.2±0.995</td>
<td>16.73±1.51</td>
<td>0.58±0.083</td>
</tr>
</tbody>
</table>

### Table (5): Hemoglobin Electrophoresis Parameters:
### Groups of Hemoglobin

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hemoglobin Adult (Hb A) (%)</th>
<th>Normal Adult Hemoglobin (Hb A2) %</th>
<th>Hb C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>97.23±0.618</td>
<td>2.77±0.618</td>
<td>0±0</td>
</tr>
<tr>
<td>PHZ</td>
<td>96.91±0.981</td>
<td>2.59±1.168</td>
<td>0.5±0.447</td>
</tr>
<tr>
<td>Molasses</td>
<td>97.07±1.146</td>
<td>2.97±1.136</td>
<td>0±0</td>
</tr>
<tr>
<td>White Peel</td>
<td>96.38±1.311</td>
<td>3.66±1.343</td>
<td>0±0</td>
</tr>
<tr>
<td>Red Peel</td>
<td>96.97±1.755</td>
<td>3.84±1.701</td>
<td>0±0</td>
</tr>
<tr>
<td>Molasses+PHZ</td>
<td>96.79±0.936</td>
<td>3.05±0.919</td>
<td>0.42±0.394</td>
</tr>
<tr>
<td>White Peel+PHZ</td>
<td>97.47±0.97</td>
<td>2.55±0.841</td>
<td>0.17±0.164</td>
</tr>
<tr>
<td>Red Peel+PHZ</td>
<td>96.69±1.084</td>
<td>3.32±1.133</td>
<td>0.15±0.314</td>
</tr>
</tbody>
</table>

### Table (6): Iron, Erythropoietin and Glucose-6-Phosphate Dehydrogenase parameters:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Iron (ug/dl)</th>
<th>Erythropoietin(mIU/ml)</th>
<th>Glucose-6-Phosphate Dehydrogenase (G6PD) (U/gm Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>121.8±4.84</td>
<td>5.74±0.53</td>
<td>7.09±0.586</td>
</tr>
<tr>
<td>PHZ</td>
<td>408.7±51.48</td>
<td>45.09±9.55</td>
<td>2.54±0.425</td>
</tr>
<tr>
<td>Molasses</td>
<td>121.9±7.01</td>
<td>6.71±1.44</td>
<td>7.76±0.714</td>
</tr>
<tr>
<td>White Peel</td>
<td>126.9±8.04</td>
<td>6.42±1.15</td>
<td>7.76±0.378</td>
</tr>
<tr>
<td>Red Peel</td>
<td>124.8±10.58</td>
<td>6.53±0.54</td>
<td>7.32±1.721</td>
</tr>
<tr>
<td>Molasses+PHZ</td>
<td>255.4±15.69</td>
<td>28.62±4.09</td>
<td>3.31±0.412</td>
</tr>
<tr>
<td>White Peel+PHZ</td>
<td>181.5±6.02</td>
<td>19.14±2.4</td>
<td>4.61±0.328</td>
</tr>
<tr>
<td>Red Peel+PHZ</td>
<td>149±11.45</td>
<td>11.49±2.64</td>
<td>5.87±0.263</td>
</tr>
</tbody>
</table>

### Figures
Figure 1

Effect of oral administration of molasses extract, white peel pomegranate extract and red peel pomegranate extract to control or phenylhydrazine (PHZ) rats both at 25 mg/kg/day for 2 weeks on (A) Body Weight (gram). (B) Red Blood Cells (mill/cmm). (C) Mean Corpuscular Volume (MCV) (fl). (D) Mean Corpuscular Hemoglobin (MCH) (pg). (E) Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dl). (F) Red Blood Cell Distribution Width (RDW-CV) (%).
Figure 2

Effect of oral administration of molasses extract, white peel pomegranate extract and red peel pomegranate extract to control or phenylhydrazine (PHZ) rats both at 25 mg/kg/day for 2 weeks on (A) Osmotic Fragility (Initial hemolysis) (%), (B) Osmotic Fragility (Complete hemolysis) (%), (C) Red Blood Cell Survival (days), (D) Reticulocytes (%), (E) Haemoglobin Concentration (g/dl), (F) Haematocrit (%).
Figure 3

Effect of oral administration of molasses extract, white peel pomegranate extract and red peel pomegranate extract to control or phenylhydrazine (PHZ) rats both at 25 mg/kg/day for 2 weeks on (A) White Blood Cells ($\times 10^3$/cmm). (B) Neutrophils ($\times 10^3$/cmm). (C) Lymphocytes ($\times 10^3$/cmm). (D) Monocytes ($\times 10^3$/cmm). (E) Eosinophils ($\times 10^3$/cmm). (F) Basophils ($\times 10^3$/cmm).
Effect of oral administration of molasses extract, white peel pomegranate extract and red peel pomegranate extract to control or phenylhydrazine (PHZ) rats both at 25 mg/kg/day for 2 weeks on (A) Platelets($\times 10^3$/cmm). (B) Mean Platelet Volume (MPV) (fl). (C) Platelet Distribution Width (PDW) (%). (D) Plateletcrit (PCT) (%).
Figure 5

Effect of oral administration of molasses extract, white peel pomegranate extract and red peel pomegranate extract to control or phenylhydrazine (PHZ) rats both at 25 mg/kg/day for 2 weeks on hemoglobin Electrophoresis Parameters (A) Hemoglobin Adult (Hb A) (%). (B) Normal Adult Hemoglobin (Hb A2) %. (C) Hb C (%).
Figure 6

Effect of oral administration of molasses extract, white peel pomegranate extract and red peel pomegranate extract to control or phenylhydrazine (PHZ) rats both at 25 mg/kg/day for 2 weeks on (A) Iron (ug/dl). (B) Erythropoietin (mIU/ml). (C) Glucose-6-Phosphate Dehydrogenase (G6PD) (U/gm Hb).
Figure 7

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 red peel pomegranate pure-administered rat stained with H&E (X200) showing normal red and white pulps (RP and WP respectively) and central arteriole (ca) with increased deposition of red phenol pigments (asterisk). (G):
Section of spleen of white peel pomegranate pure-administered rat stained with H&E (X200) showing normal red and white pulps (RP and WP respectively) and central arteriole (ca) with no histopathological changes. (H): Section of spleen of molasses pomegranate pure - 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). (I): Section of spleen of PHZ-exposed rat treated with red peel pomegranate (Red Peel + PHZ) stained with H&E (X1000) showing good recovered splenic parenchyma with good cellularity in red and white pulps (RP and WP respectively). (J): Section of spleen of PHZ-exposed rat treated with white peel pomegranate (White Peel + PHZ) stained with H&E (X1000) showing partially-recovered splenic parenchyma in red and white pulps (RP and WP respectively) in spite of the presence of damaged and apoptotic cells (DC and ac respectively). (K): 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).