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Grape seed oil protects against phenylhydrazine-induced hemolysis in rats

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Abstract

Hemolytic anemia leads to mortality and morbidity if untreated. We evaluated the potential of Grape Seed Oil (GSO) against phenyl hydrazine (PHZ)-induced hemolytic anemia in rats. In this study, thirty male albino rats aging four weeks were randomly allocated to five groups with six rats per group: control (Group I), received a single dose of 0.1 mol/L citrate buffer. Group II, normal rats received GSO orally daily for 30 days. Groups (III-V): Rats received PHZ dissolved in dimethyl sulfoxide (DMSO) *i.p*, at dose of 10 mg/kg for 4 days. Rats in group IV and V were treated orally daily with GSO starting from day 6 for one month at 2 mL/kg body weight (bw) or 4mL/kg bw, respectively. The analysis of GSO by gas chromatography/mass spectrometry (GC/MS) showed the presence of 1-Eicosanol, Behenic alcohol, 2-Piperidinone, N-[4-bromo-n-butyl]-, 2,6-Octadiene, Pentanoic acid, 10-undecenyl ester, 1-Hexacosanol and Undec-10-ynoic acid, dodecyl ester. In addition, different amount of fatty alcohol as 1-Heptacosanol. It was concluded that GSO ameliorates PHZ-induced anemia in rats. The effect of GSO was due to its phytochemical components which enhance antioxidant capacity and anti-inflammatory effect. In addition, GSO increased ferritin storage and transferrin. We recommend the isolation of the compounds and performing mechanistic studies of their mode of action.

Introduction

Hemolysis (destruction of red blood cells) was accompanied by reduced nutrients supply and gases transport to different tissues that affect body functions.[1] Hemolysis can be caused by different exogenous or endogenous factors including heavy metals pollution, viral infections, drugs and malnutrition.[2] The consequences of hemolysis lead to anemia.[3] Phenyl Hydrazine (PHZ), is one of drug of choice for the management of polycythemia vera due to its ability for destruction of red blood cells.[4] Its limitation use is due to increased hemolysis rate.[5] When PHZ is given, it potentiates the production of reactive oxygen species (ROS) and oxidative stress.[5] The oxidative stress can induce DNA, proteins and phospholipids damage, leading to cell

damage.[6] Nowadays, functional foods rich with active natural products spotted on the management of different diseases without side effects.[7] Different natural products are widely used in folk medicine for the management of anemia. For example, apple vinegar was found to improve hematological problems and anemia in rats.[8]

The wide distribution of polyphenols in foodstuffs reflects a good health impact in different conditions, such as cardiovascular disease (CVD) and diabetes due to their antioxidant and anti-inflammatory activity. In this connection, grape is the most favorable fruit worldwide due to its good taste. The genetic variability among different varieties produced different flavor and color.[9] The grape seeds were previously investigated for their antioxidant activity in protection against ROS and oxidative stress.[10] Grape seeds contain bioactive components such as phenolics and flavonoids.[11] Grape seeds were used as anti-inflammatory, immunomodulatory, antimicrobial and for the management of dyslipidemia.[12] It was reported that grape seed extract was found to protect against myocardial ischemia caused by ROS and brain damage in ischemia experimental animal model.[13] In addition, it can be used in cosmetic products as antiaging due to its phenolic content that is distributed in the fruit, peels, and pulp.[14]

There is no any study investigating the use of grape seed oil (GSO) in the treatment of hemolytic anemia. The rationale of the current study was designed to evaluate the role of GSO in protection against PHZ-induced hemolytic anemia in rats.

Materials and Methods

Identification of GSO content by GC/MS

Grape seed oil was purchased from Jeddah Oil market, purity (98.2%). The identification of GSO components was carried out using gas chromatography/mass spectrometer (GC/MS), (Agilent Technologies 7890B, Santa Clara, USA). The GC was equipped with a 20 m × 9 × 0.18 mm column. One mL of GSO was dissolved in one mL acetonitrile High Performance Liquid Chromatography (HPLC) grade, then 20 µL were injected into GC column (Agilent 19091S-433UI, HP-5ms Ultra Inert: 30 m × 250 µm × 0.25 µm). The flow rate of helium gas was maintained at 1 mL/min.[3] GSO components were identified based on retention time relative to library standard (National Institute of Standards and Technology, Gaithersburg, MD, USA).

Determination of phenolics and flavonoids content in GSO

Total phenolics were determined by using Folin–Ciocalteu reagent solution (Sigma-Aldrich, NY, USA) colorimetric methods as described by Laaroussi *et al.*[16] While The flavonoids content was evaluated using UNICO UV-2100 spectrophotometer (New Jersey, USA) according to the method mentioned by Kong *et al.*[17]

Assay of total antioxidant activity

The total antioxidant activity of GSO was quantified by using phospho molybdenum according to Zengin *et al.*[18] Briefly, 50 µL of sample was mixed with 2 mL of ph.ospho molybdenum, mixed well and incubated at 95 ˚C for 30 minutes. The optical density was measured at 695 nm using UNICO UV-2100 spectrophotometer, New Jersey, USA.

Assay of radical scavenging activity

The radical scavenging potential was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution according to Miguel *et al.*[19] Briefly, 25 µL of sample was mixed with 2 mL of DPPH solution. Optical density was read at 517 nm for 1 h using UNICO UV-2100 spectrophotometer, New Jersey, USA. The Percentage of Inhibition (PI) of DPPH radical was determined based on the percentage inhibition of free radical:

$$PI(\%) = A\ control - A\ sample / A\ control \times 100$$

Where: A: Absorbance.

Experimental design

The handling of animals was done according to Ethical Committee of King Abdulaziz University (KAU-344:10/4/2025). Thirty male albino rats weighting 120±20 g were obtained from animal house, King Fahad Medical Research Center, KAU (Jeddah). The animals were distributed in clean plastic cages with free foods and water for one week adaptation before experiment. Rats were randomly grouped into five groups (each 6 rats): control (Group I), received a single dose of 0.1 mol/L citrate buffer. Group II rats received GSO orally daily for 30 days. Groups (III-V):

Rats received PHZ dissolved in dimethyl sulfoxide (DMSO) *i.p*, at a dose of 10 mg/kg for 4 days; the dose of PHZ was given according to Lee *et al.*[20] Rats in group IV and V were treated orally daily with GSO starting from day 6 for one month at 2 mL/kg body weight (bw) or 4 mL/kg bw, respectively; the doses were given according to Berahmand *et al.*[9] At the end of experiment, rats were fasted overnight. Blood was collected directly from the heart. All kits were obtained from ABCAM, Cambridge UK. Hemoglobin was determined by Colorimetric method using kit Cat # (ab234046), iron by colorimetric assay using kit Cat# ab83366, transferrin by enzyme linked immunosorbent assay (ELISA) kit #ab137993, ferritin by ELISA kit cat # 157732, erythropoietin by ELISA kit cat # 274398, malondialdhyde by ELISA kit # ab238537, superoxide dismutase by colorimetric kit # ab65354, reduced glutathione by fluorometric assay kit # ab65322 , tumor necrosis factor (TNF-α) by ELISA kit # ab100784 and interleukin-6 (IL-6) levels by ELISA kit # 234570.

Statistical analysis

SPSS version 24 for Windows, was used for all data analysis. Data were compared using one-way analysis of variance (ANOVA), followed by least significant difference post hoc multiple comparison test. The data was significant when the p value was ≤ 0.05.

Results

The analysis of GSO by GC/MS (Table 1) showed many active ingredients including 1-Eicosanol, Behenic alcohol, 2-Piperidinone, N-[4-bromo-n-butyl]-, 2,6-Octadiene, Pentanoic acid, 10-undecenyl ester, 1-Hexacosanol and Undec-10-ynoic acid, dodecyl ester. Most of these are unsaturated compounds. Other phytosterols were detected as decan derivatives. The GSO revealed the presence of seventeen chemical constituents, most of them fatty acids derivatives. Data obtained showed that the total grape seed oil phenolic and flavonoid contents, total antioxidant activity and radical scavenger activity were 143±10.2 mg Gallic Acid Equivalent (GAE)/100 mL, 33±2.9 mg Quercetin (QE)/100 mL, 55±4.9 mg Ascorbic Acid Equivalent (AAE)/100 mL, and 6.4±0.65 mL, respectively. Data in Figure 1a, b, and c show a significant reduction ($p<0.001$) in the levels of blood hemoglobin, serum ferritin and transferrin in rats injected with PHZ compared with normal rats. However, treatment with GSO at doses 2 mL or 4

mL kg bw showed improvement in blood hemoglobin, serum ferritin and transferrin compared with untreated rats ($p<0.001$). On the other hand, erythropoietin level did not show any significant changes among different groups injected with PHZ or treated with GSO versus control (Figure 1d). The oxidative stress markers are presented in Figure 2a, b, and c and showed that rats injected with PHZ had a significant elevation in the level of MDA ($p<0.001$) concomitant with a significant decrease in the activity of SOD and reduced glutathione compared with control. Treatment with GSO (2 or 4 mL) showed a significant reduction in MDA level and a significant elevation in reduced glutathione level and activity of SOD compared with untreated rats. Obtained data shown in Figure 3a and b revealed a significant elevation ($p<0.001$) in the levels of serum IL-6 and TNF-α in rats injected with PHZ compared with control rats, while they returned to about normal in treatment with GSO (2 mL or 4 mL) compared with untreated. The effect was dose dependent.

Discussion

Iron deficiency is the main cause of anemia in both sexes. In addition, infections, malnutrition like folate deficiency, toxicity or inherited disorders such as hemoglobinopathies are major factors.[21] Hemolytic anemia led to reduced hemoglobin and cause different abnormalities. As shown in this study there was a significant decrease in hemoglobin level in rats injected with PHZ compared to the control rats, in accordance with a previous study.[22] This is due to the toxic effect of PHZ, that caused lipid peroxidation of phospholipids of membrane of blood cells.[22] These changes in hemoglobin were normalized by administration of GSO. Another study reported that Grape seeds contain proanthocyanidins (phenols) that have antioxidant potential important for health for collagen formation, elasticity, and flexibility.[23] Data obtained showed that serum transferrin and ferritin were significantly decreased in rats injected with PHZ while they were restored by treatment with GSO. The effect was dose dependent. However, obtained data did not show significant changes in erythropoietin level in PHZ-injected or GSO-treated rats compared with control group. The phytochemical content of GSO as presented by the presence of 1-Eicosanol, Behenic alcohol, 2-Piperidinone, N-[4-bromo-n-butyl]-, 2,6-Octadiene, Pentanoic acid, 10-undecenyl ester, 1-Hexacosanol and Undec-10-ynoic acid, dodecyl ester, may be related to the antianemia effect. In the current study, the injection of PHZ into rats showed a

significant elevation in serum MDA, reduced glutathione and SOD compared with normal rats. These data may be due to oxidative stress. It was reported that PHZ caused an elevation in the levels of plasma malondialdehyde as a marker for lipid peroxidation.[20] Previous study reported that MDA is a product of lipid membrane peroxidation due to the high free radicals level; thus MDA was estimated as a marker for oxidative stress.[24] Phenyl hydrazine caused MDA elevation versus control. In addition, a significant reduction in MDA was observed after feeding beetroot pomace biscuit for 28 days due to its high antioxidant power of phenolics in beetroot.[25] SOD and reduced glutathione represent the antioxidant system, and showed a significant reduction after PHZ injection, then reversed to normal with GSO treatment. A previous study showed that food rich in polyphenols affects iron absorption but improves erythropoiesis by increasing rate of hemoglobin synthesis.[26] Due to its antioxidant and anti-inflammatory activity, GSO may affect erythropoiesis. Chronic diseases are generally accompanied by inflammation pathway. For these reasons foods rich in anti-inflammatory compounds are beneficial for the control of chronic diseases. Olas *et al.*[27] reported that GSO lowered platelet adhesion. Also, Sano *et al.*[28] found that grape seed extract reduced oxidized low density lipoprotein (LDL-c) as cardioprotective effect of GSO. In addition, GSO can inhibit the release of arachidonic acid that is responsible for the production of inflammatory response.[29] Also, GSO showed a neuroprotective effect against carbon tetrachloride (CCl_4)-induced brain injury in γ-irradiated rats via scavenge free radicals, inhibits inflammatory mediators, enhances the antioxidant enzymes capacity, and inhibits nitic oxide synthetase.[30] It was reported that flavonoids, that have capacity to inhibit ROS, free radicals scavenging and iron homeostasis, are promising as iron chelators agents.[31]

Conclusions

GSO ameliorates PHZ-induced anemia in rats. This effect may be due to its high contents of phytochemicals that enhance antioxidant (SOD and GSH) and inhibit the release of inflammatory mediators (IL-6, TNF-α). In addition, GSO improves ferritin and transferrin for storage and transport of iron. We recommend the isolation of the compounds and performing mechanistic studies of their mode of action.

References

1. Barcellini W, Fattizzo B. Clinical applications of hemolytic markers in the differential diagnosis and management of hemolytic anemia. Dis Mark 2015;2015:635670.
2. Van Avondt K, Nur E, Zeerleder S. Mechanisms of haemolysis-induced kidney injury. Nat Rev Nephrol 2019;15:671–92.
3. Barbaryan A, Iyinagoro C, Nwankwo N, et al. Ibuprofen-induced hemolytic anemia. Case Rep Hematol 2013:2013:142865.
4. Berger J. Phenylhydrazine haemotoxicity. J Appl Biomed 2007;5:125–30.
5. Gniadek, TJ, Arndt PA, Leger RM, et al. Drug-induced immune hemolytic anemia associated with anti-vancomycin complicated by a Paraben antibody. Transf 2018;58:181–8.
6. Fibach E, Rachmilewitz E. The role of oxidative stress in hemolytic anemia. Curr Mol Med 2008;8:609–19.
7. Laaroussi H, Bakour M, Ousaaid D, et al. Protective effect of honey and propolis against gentamicin-induced oxidative stress and hepatorenal damages. Oxid Med Cell Longev 2021:2021:9719906.
8. Ousaaid D, El Ghouizi A, Laaroussi H, et al. Anti-anemic effect of antioxidant-rich apple vinegar against phenylhydrazine-induced hemolytic anemia in rats. Life 2022;12:1-14.
9. Berahmand F, Anoush G, Hosseini MJ, Anoush M. Grape seed oil as a natural therapy in male rats with Alzheimer's diseases. Adv Pharm Bull 2020;10:430-6.
10. Abe LT, Da Mota RV, Lajolo FM, et al. Compostos fenólicos e capacidade antioxidante de cultivares de uvas *Vitis labrusca* L. e *Vitis vinifera* L. [Phenolic compounds and antioxidant activity of *Vitis labrusca* L. and *Vitis vinifera* L. cultivars.] Food Sci Technol 2007;27:394–400.
11. Belviranlı M, Gökbel H, Okudan N, Başaralı K. Effects of grape seed extract supplementation on exercise-induced oxidative stress in rats. Br J Nutr 2012;2:249-56.
12. Garavaglia J, Markoski MM, Oliveira A, Marcadenti A. Grape seed oil compounds: Biological and chemical actions for health. Nutr Metab Insights 2016;9:59–64.

13. Pilehvar A, Tabrizi BA, Javadi A. The effect of grape seeds oil on lipid content of serum in rats. Adv Biores 2013;4:21-5.
14. Revilla E, Alonso E, Kovac V. The content of catechins and procyanidins in grape and wines as affected by agroecological factors and technological practices. In T. R. Watkins (Ed.), 1997. Wine: nutritional and therapeutic benefits (pp. 69-80). Washington: American Chemical Society.
15. Feng Y, Liu YM, Fratkins JD, et al. Grape seed extract suppresses lipid peroxidation and reduces hypoxic ischemic brain injury in neonatal rats. Brain Res Bull 2005;66:120–7.
16. Laaroussi H, Bouddine T, Bakour M, et al. Physicochemical properties, mineral content, antioxidant activities, and microbiological quality of *Bupleurum spinosum* Gouan honey from the Middle Atlas in Morocco. J Food Qual 2020;2020:7609454.
17. Kong KW, Mat-Junit S, Aminudin N, et al. Antioxidant activities and polyphenolics from the shoots of *Barringtonia racemosa* (L.) Spreng in a polar to apolar medium system. Food Chem 2012;134:324–32.
18. Miguel MdG, Doughmi O, Aazza S, et al. Antioxidant, anti-Inflammatory and acetylcholinesterase inhibitory activities of propolis from different regions of Morocco. Food Sci Biotechnol 2014;23:313–22.
19. Zengin G, Arkan T, Aktumsek A, et al. A Study on antioxidant capacities and fatty acid compositions of two *Daphne* species from Turkey: New sources of antioxidants and essential fatty acids. J Food Biochem 2013;37:646–53.
20. Lee HW, Kim H, Ryuk JA, et al. Hemopoietic effect of extracts from constituent herbal medicines of Samul-Tang on phenylhydrazine-induced hemolytic anemia in rats. Int J Clin Exp Pathol 2014;7:6179-85.
21. WHO. The global prevalence of anemia in 2011. Retrieved from Geneva: World Health Organization. 2015.
22. Swem TF, Aba PE, Udem SC. Effect of hydro-methanol stem bark extract of *Burkea africana* on erythrocyte osmotic fragility and haematological parameters in acetaminophen-poisoned rats. Clin Phytosci 2020;6:65.
23. Shi J, Yu J, Pohorly JE, et al. Polyphenolics in grape seeds-biochemistry and functionality. J Med Food 2003;6:291–9.

24. Peker EGG, Ebegil M, Balabanli KB, Cevher ŞC. Evaluation of serum malondialdehyde and nitric oxide levels in patients with cystic echinococcosis. G U J Sci 2018;31:700-5.
25. Abdo EM, El-Sayed Shaltout O, El-Sohaimy S, et al. Effect of functional beetroot pomace biscuit on phenylhydrazine induced anemia in albino rats: Hematological and blood biochemical analysis. J Funct Foods 2021;78:104385.
26. Xu T, Zhang X, Liu Y, et al. Effects of dietary polyphenol supplementation on iron status and erythropoiesis: a systematic review and meta-analysis of randomized controlled trials. Am J Clin Nutr 2021;114:780-93.
27. Olas B, Wachowicz B, Stochmal A, et al. The polyphenol-rich extract from grape seeds inhibits platelet signaling pathways triggered by both proteolytic and non-proteolytic agonists. Platelets 2012;23:282–9.
28. Sano A, Uchida R, Saito M, et al. Beneficial effects of grape seed extract on malondialdehyde-modified LDL. J Nutr Sci Vitaminol (Tokyo) 2007;53:174–82.
29. Santangelo C, Varì R, Scazzocchio B, et al. Poly phenols, intracellular signalling and inflammation. Ann Ist Super Sanita 2007;43:394–405.
30. Ismail AF, Moawed FS, Mohamed MA. Protective mechanism of grape seed oil on carbon tetrachloride-induced brain damage in γ-irradiated rats. J Photochem Photobiol B 2015;153:317–23.
31. Lesjak, M, Srai SKS .Role of dietary flavonoids in iron homeostasis. Pharmaceuticals 2019;12:1-21.

Table 1. Identification of grape seed oil components analyzed by gas chromatography/mass spectrum. PK, peak; RT, retention time.

PK	RT	Area Pct	Compound name /Library/ID	Ref	CAS
1	10.3873	0.8417	5-Tridecene, (Z)-	193960	1000406-16-4

2	10.5589	0.3022	1,2-Dioxolan-3-one, 5,5-diethyl-4-methylene-	29785	136066-33-6
3	10.9137	0.7198	2,6-Octadiene	6019	004974-27-0
4	11.0739	0.4376	Pentanoic acid, 10-undecenyl ester	115375	1000159-93-4
5	11.4115	1.3583	Dodecane, 1,2-dibromo-	236483	1000309-70-8
6	11.5775	0.8146	Pentanoic acid, 10-undecenyl ester	115375	1000159-93-4
7	11.8979	2.6102	n-Nonadecanol-1	95915	195194-80-0
8	12.0524	1.7582	1-Eicosanol	39655	007333-23-5
9	12.3614	6.8283	Behenic alcohol	11808	004316-65-8
10	12.5101	4.9871	n-Tetracosanol-1	102562	000629-80-1
11	12.6761	6.6978	1-Hexacosanol	128815	1000131-10-2
12	12.8077	11.5599	1-Heptacosanol	237628	1000309-38-3
13	12.945	11.8456	E-2-Octadecadecen-1-ol	128815	1000131-10-2
14	13.0881	13.7901	2-Piperidinone, N-[4-bromo-n-butyl]-	237628	1000309-38-3
15	13.2254	13.2049	Undec-10-ynoic acid, dodecyl ester	128815	1000131-10-2
16	13.3455	9.1545	Oxalic acid, isobutyl octadecyl ester	237628	1000309-38-3

17	13.4772	13.0891	Dodecane, 1,2-dibromo	171288	006175-11-7

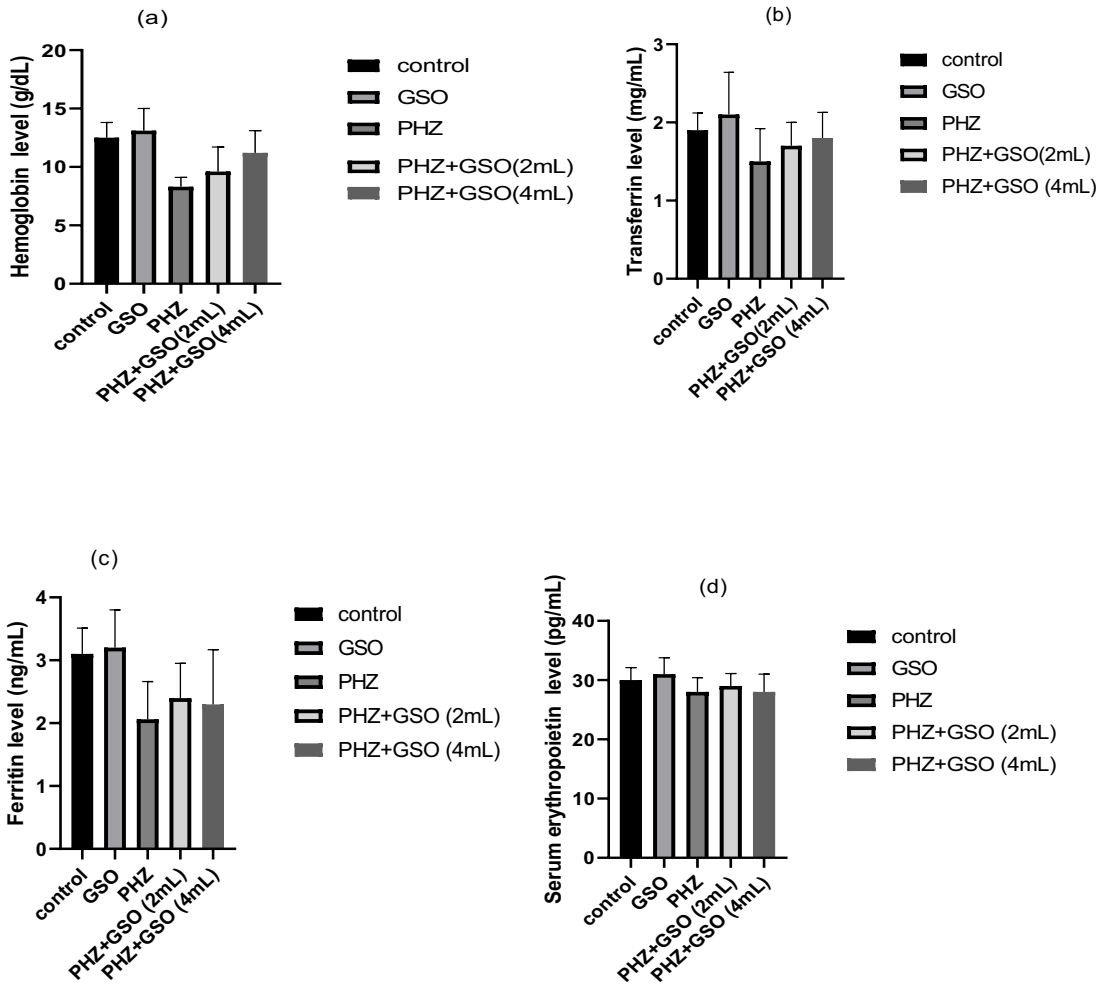

Figure 1. a) Hemoglobin levels in all studied groups; b) Transferrin level in all studied groups; c) Serum ferritin level in all studied groups; d) Serum erythropoietin level in all studied groups. GSO, grape seed oil; PHZ, phenyl hydrazine.

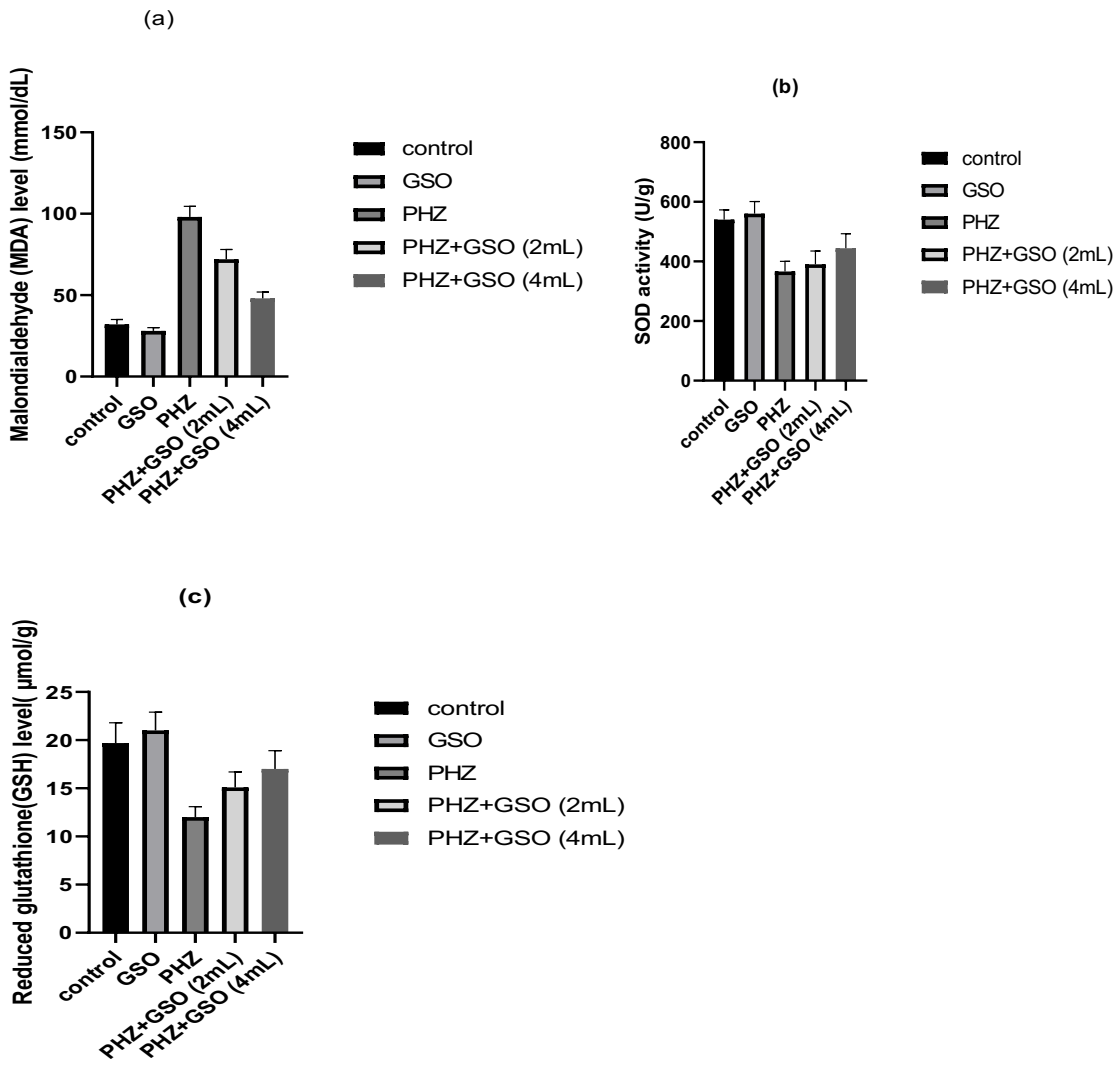

Figure 2. a) Serum malondialdehyde (MDA) level in all studied groups; b) Serum superoxide (SOD) activity in all studied groups; c) Serum reduced glutathione level (GSH) in all studied groups. GSO, grape seed oil; PHZ, phenyl hydrazine.

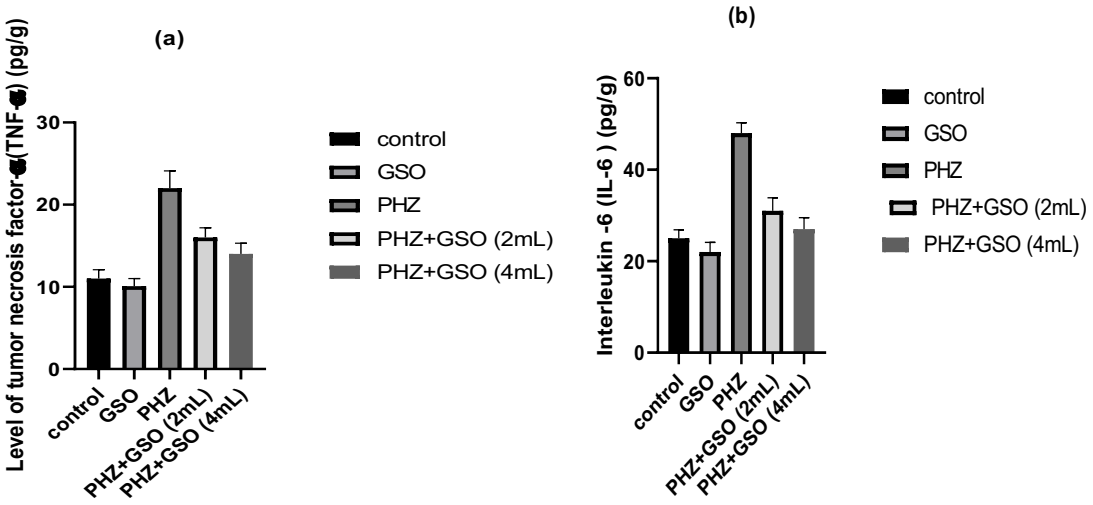

Figure 3. a) Serum level of tumor necrosis factor (TNF-α) in all studied groups; b) Serum interleukin-6 (IL-6) level in all studied groups. GSO, grape seed oil; PHZ, Phenyl hydrazine.