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Original article

The Protective Effect of *Pistacia vera* Pericarp on Kidney Function in Rats with Hemolytic Anemia

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Abstract

Background and objectives: *Pistacia vera* is known as a source of unique materials with therapeutic function such as antioxidant and nephron-protective activities. This study aimed to identify the biochemical and histopathological effects of *Pistacia vera* pericarp aqueous extract on the kidney in phenylhydrazine-induced anemia model in rats. **Methods:** Extraction of the *P. vera* pericarp was carried out by maceration technique. For animal study, the rats were studied in six groups and were exposed to phenylhydrazine for two days in the absence or presence of the extract. Renal changes were measured using biochemical and histopathological assays. The urine samples were collected in metabolic cages for total urine volume, creatinine, and 24-hour proteinuria measurement with the protein/creatinine ratio. Serum catalase, malondialdehyde and superoxide dismutase as oxidative stress markers were examined using ELISA test. **Results:** Phenylhydrazine induced kidney injuries evidenced by significant changes of urine, serum urea, creatinine levels, sodium, and potassium ions in comparison to the control group; however, the extract treatment significantly decreased kidney injuries. Administration of 80 mg/kg of the extract significantly reduced the creatinine and proteinuria in treated animals ($p<0.05$) but 160 mg/kg of extract helped the anemic animals to reduce protein and creatinine to normal levels. **Conclusion:** Twenty-four hours protein and creatinine can be used as markers of renal injuries in anemia and their regular measurement can be useful to find the risk of renal problems in anemia. These results revealed that *P. vera* pericarp administration may decrease renal injuries and dysfunction by reducing inflammation in the kidney.

Keywords: creatinine; inflammation; nephron; phenylhydrazine; *Pistacia vera*;

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Introduction

Several chemicals such as alcohol and toxins have hazards to the kidney, by disrupting

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physiological functions and causing serious problems. By 2019, renal diseases have made problems for more than 850 million individuals [1]. Thus, the search for certain factors that can diminish this burden is necessary. Common drugs have efficacy and accessibility, in addition to side effects related to their prolonged use. Therefore it is crucial to use alternative treatments [2].

There are lots of plant-derivatives and plant extracts which are often checked by using animal models for their potential nephroprotective effects. There has been a growing interest in *Pistacia vera*. It has powerful potential as a source of unique materials with therapeutic functions. *Pistacia vera* is a member of the Anacardiaceae family which is grown in different parts of the world especially in Central and West Asia such as Iran. It has been administered in herbal medicine. Iran is one of the world's major producers of pistachio [3]. According to the number of genotypes and cultivars of pistachio, Iran is one of the richest areas under the cultivation of pistachio. Different parts of *P. vera* have valuable nutrients. It is a remarkable source of fatty acids, antioxidants, and minerals. Previous studies have demonstrated pharmacological characteristics of *P. vera* [3].

It has been reported that *P. vera* has positive effects on some risk factors for cardiovascular diseases and for reducing inflammation and oxidative stress in the kidney and decreasing the inflammatory biomarkers [4].

Phenylhydrazine (PHZ) leads to haematotoxicity which causes hemolytic anemia. Hemolytic anemia can stimulate oxidative stress which leads to renal failure [5].

Iron deficiency and anemia lead to cell death via necrosis and apoptosis. Lipid peroxidation by polyunsaturated lipids in the membranes of the cells such as red blood cells can cause cell death due to the formation of free radicals and oxidative stress [6,7]. PHZ-induced anemia increases iron absorption which affects iron transport genes expression. Hepcidin gene expression is down-regulated while the expression level of Dcytb, DMT1-IRE, and Ireg in the duodenum, transferrin receptor (TFR1), and hem oxygenase (HO1) in the spleen, and TFR1 in the liver are significantly up-regulated in PHZ-treated animals. anus kinase - signal transducer and activator of transcription (JAK-STAT) pathway is responsible for the maturation of RBC and PHZ modulates the erythropoietin (EPO) receptor of this pathway [8]. Additionally, PHZ makes single strand DNA fragmented [9]. PHZ also affects the immune system. It binds with autologous antibodies and macrophage can recognize the antigen-antibody which triggers phagocytosis in the spleen and liver [10].

Kidney dysfunctions in anemia can cause proteinuria. Therefore, proteinuria can be used as a marker of renal insufficiency, and routine screening for proteinuria may help find those at increased risk of renal problems. Renal injuries prevalence is high among anemia patients with proteinuria [11]. For this purpose, measuring the proteinuria from 24-h urine samples has been used as a standard method for estimation of urinary protein excretion.

The aim of the present study was investigation of the protective effect of *P. vera* pericarp aqueous extract on renal failure in rats that were exposed to hemolytic anemia induced by PHZ. Besides, it was aimed to investigate the oxidative stress markers including catalase (CAT), malondialdehyde (MDA), and superoxide dismutase (SOD) of rats with PHZ-induced anemia.

Materials and Methods

Ethical considerations

The animal experiments followed the guidelines of the Rafsanjan University of Medical Sciences (RUMS) Ethical Committee for animal experiments with animal ethical approval number: IR.RUMS.REC.1397.220 at RUMS.

Chemicals

Phenylhydrazine hydrochloride was purchased from Sigma Aldrich (USA). Ethanol and paraformaldehyde were obtained from Merck (Germany). Ketamine was purchased from Hameln, Germany. SunRed kit was obtained from Biotechnology Company (China).

Plant material

In present study fresh *Pistachia vera* L. cv *Akbari* [12] which had not been exposed to any toxic spraying was used. *Pistachia vera* var. *Aakbari* was identified by Dr. Ali Tajabadipour, a horticultural specialist, and a voucher sample (No. AK212) was kept at the Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Iran.

Pistachio pericarps were shade dried. They were kept in dark, ground and stored at -20 °C until required.

Fifty g of powdered pistachio pericarps was added to 500 mL water and kept shaking for 48 h at 130 rev/min. Then, the extract was filtered using paper filter (0.2 µm) and freeze dried by a freeze-dryer. Finally, the obtained powder was stored at -20 °C until used.

Animals

Sixty Wistar male rats with the standardized diet were used in this project. The rats were 8 weeks old weighing 200 ± 5 g. They were adapted in a standard laboratory for one week. The room temperature was 22 ± 2 °C with a humidity of 55 ±5% using ad libitum feeding. The animals were randomly divided into six groups of ten animals and received plant extract using an oral gavage after dissolving the plant extract in distilled water. They were treated as follows:

A: animals received a normal diet+water+ distilled water (at 10 mL/kg body weight) for 28 days (negative control group) [13].

B: animals received a normal diet+water+ pistachio pericarp extract (80 mg/kg body weight for 28 days; positive control group)

C: animals received phenlyhydrazine intraperitoneally for two days to induce anemia at a dose of 60 mg/kg of body weight+normal diet+ water [14].

D, E, and F: animals that received phenlyhydrazine intraperitoneally for two days to induce anemia at a dose of 60 mg/kg of body weight+normal diet (in addition of *P. vera* pericarp extract at 20 mg/kg, 80 mg/kg and 160 mg/kg body weight for 28 days, respectively)+ water [15]

The experimental duration was 28 days. The rats were placed in the metabolic cages and urine samples were collected from each animal. Twenty-four h urine samples were used for total urine volume, creatinine, protein, and protein/creatinine ratio assessment. The rats were then euthanized through intraperitoneal injection of ketamine and blood samples were taken from the heart and collected in EDTA tubes. Serum samples were also collected in plain tubes and used for biochemical analysis (Urea, Creatinine, Uric Acid, Na, and K) [16].

CAT, MDA, and SOD assay

To measure the catalase (CAT), malondialdehyde (MDA), and superoxide dismutase (SOD) levels in the samples, commercial kits from SunRed Biotechnology were used. All steps were done according to the instruction by the supplier. All samples were prepared and transferred to a 96-well ELISA plate and incubated with the antigen which immobilized to a solid surface. To omit the uncombined enzyme, the wells were completely washed. Finally, the sample was added the chromogen solutions and the color changes were measured using a spectrophotometer. The CAT, MDA, and SOD levels were also measured in each of the tissue and serum samples. The percentage change in the body weight of the experimental animals was calculated as:

Final body weight-initial body weight/final body weight×100

After sacrificing the animals, the kidneys were excised and weighed using a top-loading balance, and their relative organ weight was calculated as:

kidney weight (g)/body weight (g).

Finally, kidney tissues were fixed in 10% paraformaldehyde for 24 hours, when they were processed for histopathological analysis

Histopathological analysis

The tissues were processed by paraffin-embedded blocks. Then, the paraffin blocks were cut into 5-µm thick sections using an appropriate microtome and were stained by H&E method. Ultimately, the prepared slides were evaluated by a pathologist with a light microscope.

Statistical analysis

Statistical analysis was done by using SPSS 26.0 software (SPSS, USA). The results were demonstrated as mean ± SEM (standard error of the mean). One-way ANOVA and Tukey's multiple comparison test were used to compare the mean of the groups. $P<0.05$ was considered to be statistically significant.

Results and Discussion

In all experimental groups, the rats remained healthy and active during the experiments. The body and kidney weights of untreated, PHZ-exposed, and *P. vera* pericarp aqueous extract-treated animals were measured. The results

showed that treatment with PHZ induced a significant ($p<0.05$) body weight loss in rats in comparison to that in controls after 7 weeks. However, body and kidney weights were found to be enhanced on *P. vera* pericarp aqueous extract administration. Significant reduction in relative kidney weights were also observed in PHZ-injected animals. Administration of *P. vera* pericarp aqueous extract prevented PHZ-induced kidney and weight loss

Plasma creatinine concentration as shown in Figure 1, illustrated a significant difference ($p<0.05$). The PHZ group showed the highest concentration of creatinine when compared with other groups. Urea levels (Figure 1) were significantly different. The result demonstrated that the PHZ group showed the maximum concentration of plasma urea. The uric acid results were significantly different ($p<0.05$) in comparison with the control and the PHZ groups. On the other hand, the PHZ group showed an increase in uric acid levels compared to other groups. The erythrocytes hemolysis by PHZ produces derivatives which affect the kidney, and their accumulation causes damages to the tissue. The mean weight of kidneys was increased by using the pericarp extract of *P. vera* in comparison to the PHZ group C (Table 1). There was a marginally increase in body weights of the rats in the *P. vera* treated groups D and E in comparison to the PHZ-injected group C.

On the other hand, the relative weights of the kidneys in the rats that received the *P. vera* were significantly higher than the relative weight of kidneys in the PHZ- treated group ($p<0.05$).

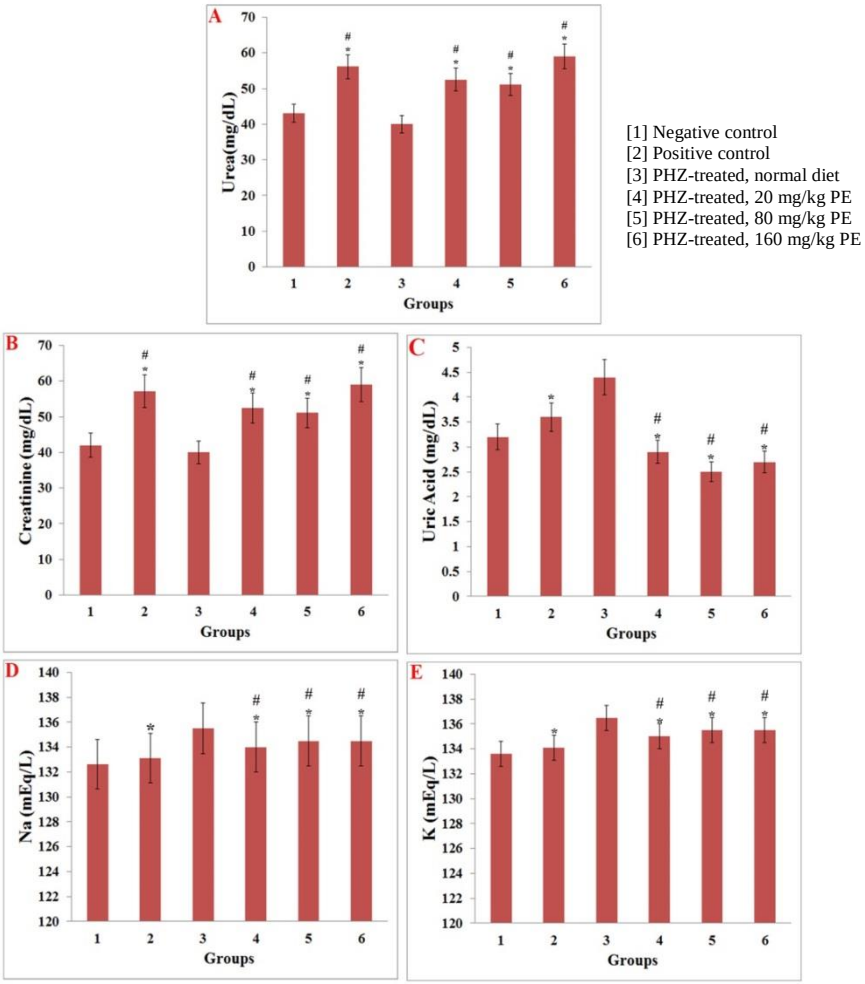

Figure 1. Effect of *Pistachia vera* pericarp extract on concentrations of urea (A), creatinine (B), uric acid (C), Na (D) and K (E) in rats with PHZ-induced anemia. Group 1: negative control and 2: positive control groups that received 10 mL/kg distilled water and 80 mg/kg pistachio pericarp extract, respectively. Groups 3, 4, 5 and 6: PHZ-treated animals that received normal diet, 20, 80 and 160 mg/kg pistachio pericarp extract, respectively. Data are expressed as mean ±SD and analyzed by one-way ANOVA followed by post hoc Tukey tests.* $p<0.05$, as compared to the PHZ group and #$p<0.05$, as compared to the control group; PHZ: phenylhydrazine; PE: pistachio pericarp extract

It is known that PHZ causes hemolytic anemia and renal injuries and in conformity with this, in the present study, increased uric acid, urea, and creatinine concentrations in PHZ-injected rats indicated nephrotoxicity. The increased levels could be the result of damages produced in kidney tubules which made changes in renal tissues in comparison to the control.

However, in the groups treated with *P. vera* pericarp extract at 20, 80, and 160 mg/kg BW, a slight reduction in the levels of uric acid concentration as well as decreased creatinine and urea concentrations were detected, and which were significantly different from the PHZ-exposed rats ($p<0.05$). The animals treated with the aqueous extract of *P. vera* pericarp (160 mg/kg BW) showed affected levels of creatinine, uric acid, and urea concentration compared with the PHZ- treated animals at $p<0.05$.

For monitoring renal function in the anemic animal model, this study aimed to evaluate the amount of proteinuria, protein/creatinine ratio, and total urine volume measured by 24 h urine collection in animals. The results showed that the creatinine and proteinuria significantly increased in PHZ-induced rats. Administration of 80 mg/Kg of *P. vera* pericarp extract significantly reduced the creatinine and proteinuria in treated animals ($p<0.05$). On the other hand, the results showed that the total volume of urine and creatinine increased in group D ($p<0.05$). Therefore, it can be the main reason for the reduction in protein/creatinine ratio in the anemic rat model in group D ($p<0.05$). Although, using *P. vera* pericarp extract could reduce the creatinine and proteinuria, at a low dose, it couldn't decline the effect of PHZ on renal function (Table 2).

Administration of 160 mg/kg of the plant extract to anemic rats caused a decline in protein and creatinine to rebound to normal levels (Table 2).

The catalase level was found to be 0.70 ± 0.10 ng/mL in group A. Mean serum CAT activity of groups D and E (0.04 ± 0.02 ng/mL and 0.03 ± 0.01 ng/mL) rats were investigated to be higher significantly ($p< 0.05$) in comparison to group C (0.02 ± 0.01 ng/mL) but they were lower than those in group A and B (Figure 2).

The serum levels of MDA in different groups are presented in figure-3B. The mean of serum levels of MDA in group A (0.71 ± 0.11 µmol/L) and group B (1.63 ± 0.21 µmol/L) were compared with group C (12.01 ± 2.53 µmol/L), group D (11.58 ± 1.63 µmol/L) group E (4.28± 1.16 µmol/L) and group F (1.20 ± 0.83 µmol/L). The difference between group C and control (A) were statistically significant ($p<0.05$). Also, mean serum levels of MDA in groups E and F were statistically significant compared to group C ($p<0.05$).

Table 1. Body, kidney, and relative kidney weight of normal rats in comparison to the experimental groups

Parameter (gr)	Group A	Group B	Group C	Group D	Group E	Group F
Body weight	243±0.54	237.3±0.87$^{*,\#}$	255.4±1.02	270.5±0.09$^{*,\#}$	272.8±0.67$^{*,\#}$	246.5±0.01$^{\#}$
Kidney weight	0.95±0.21	0.97±0.12$^{\#}$	0.92±0.11	0.96±0.11$^{\#}$	1.04±0.09$^{*,\#}$	0.93±0.12
Relative kidney weight	0.79±0.045	0.78±0.12$^{\#}$	0.63±0.35	0.79±0.28$^{\#}$	0.81±0.15$^{\#}$	0.82±0.09$^{\#}$

A, negative control and B, positive controls received 10 mL/kg distilled water and 80 mg/kg pistachio pericarp extract, respectively. C, D, E and F: PHZ-treated animals that received normal diet, 20, and 160 mg/kg pistachio pericarp extract, respectively. Values with * and $^{\#}$ indicate significant difference ($p<0.05$) between experimental groups compared to the control and PHZ-injected rats, respectively. PHZ: phenylhydrazine

Table 2. Urine collection characteristics by 24 hours

Groups	RBC (×10^{6}µL)	HB (g/dL)	Protein (mg/24h)	Volume (mL)	Creatinine	Protein/ Creatinine
A	8.72±0.4	16.07±0.91	4.98±0.79	4.55±1.05	3.70±0.66	1.34
B	8.31±0.32*	15.75±1.25*	2.29±0.10*	3.05±0.53*	2.15±0.37*	1.07
C	3.98±0.37	11.58±0.71	6.44±0.01*	4.10±0.20	5.48±1.05*	1.17
D	7.91±0.31*	15.44±1.14*	3.93±1.11	5.00±1.56*	5.67±1.86*	0.69*
E	7.44±0.5*	14.83±1.31*	2.62±0.15*	2.45±0.07*	2.06±0.44*	1.27
F	7.99±0.74*	15.06±0.89*	5.76±1.52	4.85±0.92	3.46±0.93	1.66

A, negative control and B, positive controls received 10 mL/kg distilled water and 80 mg/kg pistachio pericarp extract, respectively. C, D, E and F: PHZ-treated animals that received normal diet, 20, 80 and 160 mg/kg pistachio pericarp extract, respectively; Data are expressed as Mean±SEM (n=10). Significant differences were indicated by *$p < 0.05$.

Mean serum MDA levels of groups D and E were significantly ($p<0.05$) higher than those of control rats in group A.

Based on the data of the present study, the generation of free radicals due to lipid peroxidation was significantly elevated in PHZ-induced anemia rats. The activity of SOD in the serum is shown in figure-3C. Mean serum levels of SOD in group A (0.70 ± 0.10 ng/mL) are compared with PHZ-induced animals in group C, D, E, and F. Mean serum levels of SOD in groups C, D, E, and F were significantly lower than the control ($p<0.05$). On the other hand, after consumption of the plant extract showed significantly higher levels in groups D and E in comparison to group C ($p<0.05$).

In the PHZ-treated group, while tissue MDA (16.94±0.94) (nmol/mg) level and SOD (71.23±1.64) (U/g protein) and CAT (61.32±0.98) (U/g protein) activities increased, after administration of plant extract at a dose of 160 mg/kg the MAD (12.81±1.09) (nmol/mg) levels and SOD (55.61±1.08) (U/g protein) and CAT (49.28±1.73) (U/g protein) activities were significantly reduced ($p<0.05$) (Table 3).

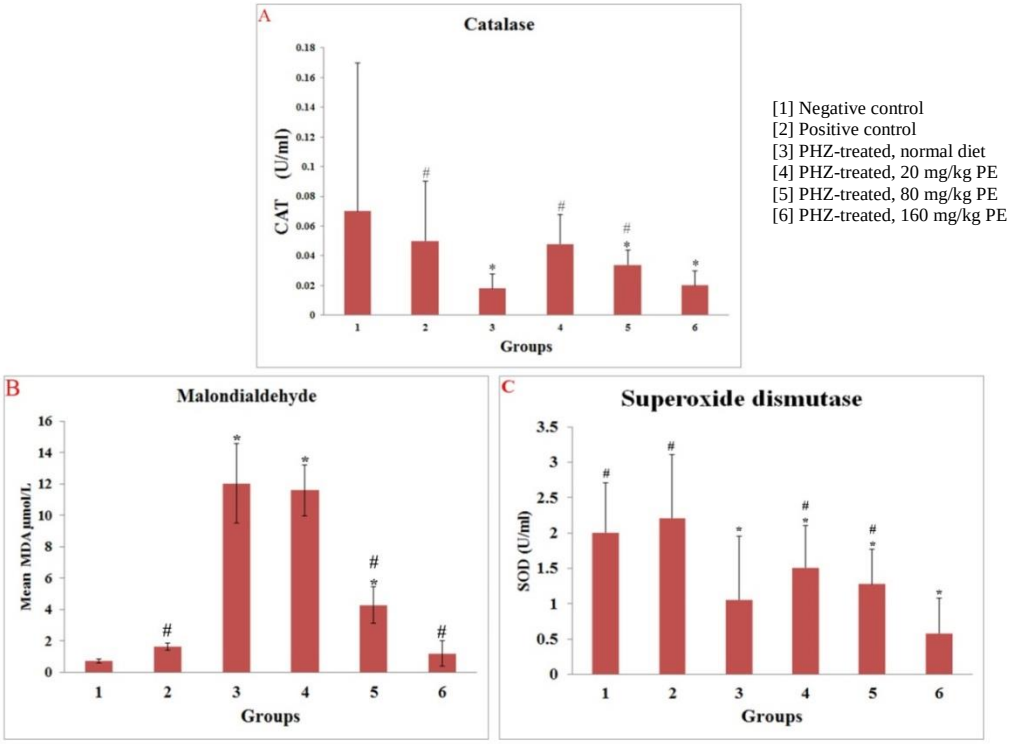

Figure 2. Levels of mean serum A) catalase, B) malondialdehyde, and C) superoxide dismutase in healthy control rats compared with PHZ-induced and *Pistachia vera* pericarp extract treated animals; groups 1: negative control and 2: positive control received 10 mL/kg distilled water and 80 mg/kg pistachio pericarp extract, respectively. Groups 3, 4, 5 and 6: PHZ-treated animals received normal diet, 20, 80 and 160 mg/kg pistachio pericarp extract, respectively. Values are expressed as mean ± SD; #: significant difference at $p<0.05$ of groups compared to PHZ-induced group; *: significant difference at $p<0.05$ of groups compared to control; CAT: catalase; MDA: malondialdehyde; SOD: superoxide dismutase

Table 3. Levels of MDA, SOD, and CAT in renal tissue

Groups	MDA (nmol/mg)	SOD (U/g protein)	CAT (U/g protein)
A	10.78±1.36	51.84±1.51	41.24±1.03
B	10.56±1.72	52.31±0.84	42.01±0.86
C	16.94±0.94	71.23±1.64	61.32±0.98
D	15.78±1.64	68.25±0.96	59.23±0.48
E	14.86±1.95	61.31±1.42	53.27±1.07
F	12.81±1.09*	55.61±1.08*	49.28±1.73*

MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase enzyme; A, negative control and B, positive control groups received 10 mL/kg distilled water and 80 mg/kg pistachio pericarp extract, respectively. C, D, E and F: PHZ-treated animals received normal diet, 20, 80 and 160 mg/kg pistachio pericarp extract, respectively. Data are expressed as Mean±SEM (n=10); significant differences were indicated by *$p < 0.05$ as compared with the PHZ-treated group.

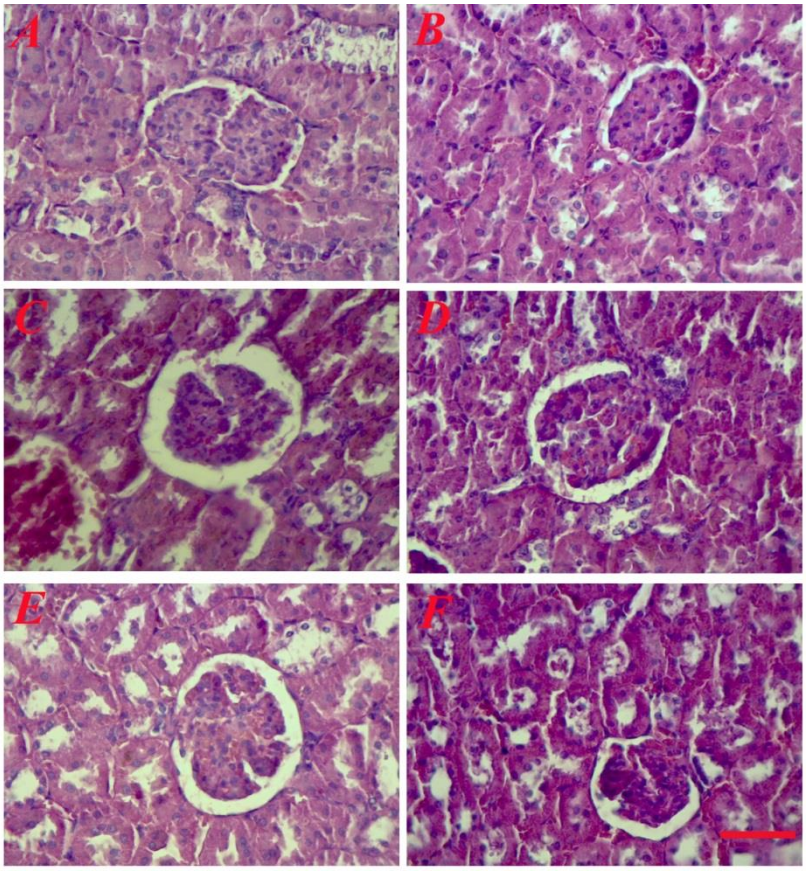

Figure 3. Histopathological findings in the kidney of rat after PHZ administration (hematoxylin and eosin staining); A: negative control group shows normal glomeruli and tubular structures; B: positive control group; C: PHZ group (anemic control group); ascular congestion is present in this group but glomeruli and tubular structure are normal; D, E and F: PHZ groups treated with pistachio pericarp extract at 20 mg/kg, 80 mg/kg and 160 mg/kg body weight for 28 days that show normal glomeruli and tubular structures (Scale bar = 200 μm.); PHZ: phenylhydrazine

Multiple cut sections from the kidney in control and treated groups with the extract show no significant pathologic changes and only prominent vascular congestion is identified in rats treated with phenylhydrazine. A and B groups had glomerular and normal tubular structure, following treatment of rats with *P. vera* pericarp extract. In D, E, and F groups, the renal tissue injuries were significantly decreased in comparison to the PHZ group (C). The *P. vera* pericarp extract caused significant reduction in cellular necrosis, scattering of cells into the tubule lumen and vacuolization of renal epithelial cells (Figure 3).

The present study was aimed to study the regenerative and protective effects of *P. vera* pericarp extract against renal failures. Erythrocyte destruction occurs after PHZ treatment. Dvanajscak et al., indicated that hemolysis-associated hemoglobin cast nephropathy is the main cause of intravascular hemolysis which can influence treatment [17]. An increase in hemolysis and further injuries has been observed in the pathogenesis of kidneys. The concentration of urea and creatinine concentrations are the most useful markers in serum and plasma and glomerular filtration rate and kidney functions [18]. The mutations in renin has caused anemia as well as hyperuricemia and mild hyperkalemia, because of the reduction in the excretion of renal urate [19]. Merle et al., have reported considerable changes in mRNA levels of the genes which are related to tubular damage and vascular inflammation in kidneys of PHZ-exposed mice. Additionally, PHZ-treated mice have also showed some tubular injuries [20]. The kidney is subjected to various kinds of toxic substances such as chemicals, alcohol, and drugs, which is a big problem worldwide. Several studies have confirmed the use of plant extracts

for reducing renal failure [21].

PHZ itself causes inflammatory changes in the kidney. Although the kidney mentions only a small part of the body weight, it receives about one-half of the circulating blood volume and has a great capacity of urinary concentration, which confirms the concentrate of toxins by renal cells, in comparison to other organs [22].

Ekweogu et al., demonstrated the consumption of *S. aethiopicum* leaf as an anti-anaemic agent with a wide range of safety and reno-protective potentials [23].

The results of the present study indicated that extracts of *P. vera* had protective effects on kidney which might be exclusively related to its antioxidant activity. In addition, during the experiments, no symptoms of renal toxicity had been found after using *P. vera* pericarp extract at dosages of 20-160 mg/kg. Therefore, it was hypothesized that *P. vera* pericarp extract may contain antitoxic substances which can repair kidney failures caused by PHZ based on the histologic appearance of this organ.

Previous studies demonstrated that, 3-(8-pentadecenyl)-phenol, 3-(10-pentadecenyl)-phenol, 3-pentadecyl-phenol and 3-(10-eptadecenyl)-phenol as the most abundant cardanols in pistachio kernel [24]. Arena et al., found phytosterols, fatty acids, nthocyanins, chlorophylls, and xanthophylls in pistachio kernel [25]. Another study reported carotenoid, chlorophyll, and chlorophyll-derived compounds in pistachio kernels as well as the anacardic acids in the outer green shell [26].

PHZ has previously demonstrated to induce toxicity [27]. The renal damages of PHZ were well recorded and the histopathological findings were comparable to the previous literature. Accordingly, the renal proximal tubules are notable transformation sites and sensitive to chemicals because they have biotransformation enzymes from the cytochrome P450 family. They moderate the establishment of reactive intermediates and toxic metabolites [28]. The kidneys are sensitized to substances that regulate vascular tone and PHZ-induced vasoconstriction makes an ischemia, which causes cellular damage by destroying the integrity of the membrane [29].

The ischemic injury causes tubular necrosis of epithelial cells in the basement membrane, forming clusters that can decrease the filtration rate, induce intraluminal pressure, and reduce the glomerular filtration rate (GFR). Therefore, a return leakage of the filtrate to the interstice happens, which further GFR reduction [30]. All these incidents can cause functional nephrons overload and renal failure[29].

The findings of group C illustrated the PHZ-induced nephrotoxicity. Contrary to this group, other groups revealed nephroprotective effects of *P. vera* against PHZ-induced injury and were demonstrated to be safe up to 160 mg/kg (Figure 3). In a previous study it was proposed that *P. vera* consumption may reduce renal injuries and structural failure by reduction of oxidative stress [31].

Potassium (K) is the most intracellular cation which is crucial to the maintenance of cell membrane potential and cellular functions by Na-K-ATPase. On the other hand, in response to increased potassium in the serum, kidneys distal renal tubular sodium delivery and tubular fluid flow eliminate the intake of potassium. Kidneys play an important role in the homeostasis of potassium [32].

The results from the PHZ group illustrated that kidney recovery can be achieved after administration of *P. vera* pericarp extract. In fact, the epithelium of proximal tubules in the kidneys can also be regenerated through proliferation, differentiation, and migration process of the cells that survive. They regenerated the layer of tubular cells. After 28 days, the tubules demonstrated a completely functional and normal epithelium. Our results were consistent with these results, and PHZ can be totally eliminated from the body. The inflammatory effects remain, and probably more time is needed for complete regeneration. The observed basophils in the tubules of the kidney may reveal a sign of tubular recovery. These cells showed more mitotic activity and development in the regenerate tissue after about one week of necrosis in the tubular cells, but the basement membrane stayed intact [33]. However, in the experimental group, basophils persevered in the kidney after 28 days, showing the complete epithelial lining; epithelial cell regeneration persisted, as also observed in renal tubule necrosis caused by PHZ [34].

However, 24-hour urine samples were applied for complementary biochemical tests. The results of the present study demonstrated the positive effects of the consumption of 80 mg/Kg of plant extract on proteinuria and creatinine. To control the urine concentration, and confirmed data, it is necessary to normalize the urine protein and

creatinine concentration. Moreover, blood samples and spot urine collection may also be useful to measure creatinine and protein levels, especially when 24-hour urine has been ordered.
24 h biomarkers such as creatinine and proteinuria could be estimated for morning renal functions. 24-hour urine collection is a useful and practical alternative to spot urine collections to evaluate some markers of renal damage and related to anemia.
Furthermore, the catalase reduction activity in PHZ-induced rats in comparison to control animals could be due to the necessity of iron for the synthesis of catalase [35].
RBCs antioxidant capacity was found to be involved in PHZ-induced anemia rats with a significant reduction in antioxidant activities of enzymes like SOD and CAT. In this study, the reduction in MDA levels of renal tissue and also the decrease in renal SOD and CAT activities demonstrated the role of oxidative mechanism affected by *P. vera* extract via reducing tissue injury in animal kidneys. The decrease in the activity of SOD can be due to CAT activity reduction, which acts through a feedback inhibition mechanism. CAT activity reduction causes hydrogen peroxide accumulation which leads to SOD activity inhibition [36]. Türkoğlu et al. studied the effects of *Pistacia terebinthus* extract on lipid peroxidation and SOD enzyme activity. It was found that SOD enzyme activity significantly changed in the blood after *Pistacia terebinthus* extract consumption in rats [37].
Previous studies reported that *P. vera* pericarp extract showed suitable safety profile and was well-tolerated. In an animal model, *P. vera* pericarp extract has reduced the severity of kidney injuries, suggesting its use as a treatment for diabetes mellitus [38].
In group C, MDA levels increased, whereas the antioxidant capacity of CAT and SOD enzyme activities decreased. Therefore, anemia may lead to oxidative stress as demonstrated in the present study but *P. vera* pericarp extract can reduce this complication.
The increase of hydrogen peroxide levels may be due to changes in SOD and CAT activities.Thus, the aim of the present study was to study the relationship of the distribution of SOD and CAT activities in serum and tissues of PHZ-induced rats after consumption of *P.vera* pericarp extract as an antioxidant that inhibits oxidation.

The results of the present study indicate that *P. vera* pericarp extract has anti-anaemic potentials and shows great level of renal recovery from PHZ-induced renal injuries. The extract has nephron-protective effects and improves kidney function in PHZ-induced anemic rats. Since the renal disorder is a global health issue, more studies should be designed in longer duration to find more details about *P. vera* pericarp extract which can be used in nephrotoxicity and pathogenesis of kidney dysfunction.

Conclusion

Pistacia vera pericarp extract was well-tolerated and safe and was effective in the reduction of creatinine and proteinuria in the anemia model. The observed nephron-protective effect of *P. vera* pericarp extract against PHZ-induced nephrotoxicity in rats might be partly due to the presence of various antioxidant constituents with therapeutic properties. However, further investigation is necessary to illustrate the exact cellular and molecular signaling pathways involved in the nephron-protective properties of *P. vera* pericarp extract.

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Author contributions

Fatemeh Amin and Soudeh Khanamani Falahati-pour contributed to design and implementation of the research; Najmeh Parvaz participated in the collection of data; Nahid Askari and Sakineh Khanamani Falahati-pour contributed substantially to the drafting, writing and revising of the manuscript; Morteza Khademalhosseini contributed to the pathological analysis and interpretation of data. All authors have approved the final version of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

PHZ: phenylhydrazine; CAT: catalase; MDA: malondialdehyde; SOD: superoxide dismutase; TFR1: transferrin receptor; HO: haem oxygenase; H&E: hematoxylin and eosin; JAK-STAT: Janus kinase - signal transducer and activator of transcription; EPO: erythropoietin; GFR: glomerular filtration rate