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Pharmacognosy

Research Article

The anti-anemic effect of dried beet green in phenylhydrazine treated rats

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ABSTRACT

Phenylhydrazine (PHZ) and its derivatives were used firstly as antipyretics but the toxic action on red blood cells made their use dangerous. The aim of this study was to estimate the contents of iron and vitamins in different parts of beetroot as root and beet green (leave and stalk together) as well as their ethanol extracts. Then, the biochemical evaluation for the highest iron and vitamins contents as an anti-anemic effect against anemic rats caused by phenylhydrazine were studied. Anemia induction caused by intraperitoneal injection of phenylhydrazine at 40 mg/kg for 2 days. Rats were randomly divided into four groups (n=10/group). Group, I fed a standard diet (normal control). Group II fed standard diet contained 5% dried beet green for normal rats. Group III fed a standard diet for anemic rats. Group IV fed a standard diet containing 5% dried beet green for anemic rats. It was assayed the complete blood count (CBC): red blood cell (RBC) count, hemoglobin concentration (Hb) and haematocrit percentage (HCT) as indices measured of anaemia as well as white blood cell (WBC) count at the third day (D3), twenty-one day (D21) and forty-two day (D42). Also, the activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), the levels of serum urea, uric acid, creatinine and malondialdehyde (MDA), plasma hydrogen peroxide (H₂O₂) and total antioxidants capacity (TAC) contents were analyzed according to manufacturer's instructions. The dried beet green is a rich source of iron and vitamins contents. After feeding on standard diet containing 5% dried beet green for anemic rats, showed that levels of RBC, HB and HCT were increased and the WBC count was improved compared to anemic control group. The activity of ALT, AST, and ALP, the levels of serum urea, creatinine and MDA and plasma H₂O₂ content were significantly ameliorated while the TAC was significantly increased compared to the anemic control group. In addition, the results of the histological examination coincided with the results of blood analysis. We conclude that nutrition of 5% dried beet green for forty-two days is safe, non-toxic and reduces anemia.

Keywords: Beet green; anemic; iron; vitamins; phenylhydrazine

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Citation | Shahenda ME, Jehan BA. 2018. The anti-anemic effect of dried beet green in phenylhydrazine treated rats. Arch Pharm Sci ASU 2(2): 54-69

DOI: [10.21608/aps.2018.18735](https://doi.org/10.21608/aps.2018.18735)

Online ISSN: 2356-8380. **Print ISSN:** 2356-8399.

Received 16 September 2018. **Accepted** 31 October 2018.

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Published by: Ain Shams University, Faculty of Pharmacy

1. INTRODUCTION

Anemia is a serious global public health problem associated with an increased risk of morbidity and mortality especially in developing countries in Africa. It is characterized by the

deficiency of RBC or Hb in the blood, which results in the disturbance of oxygen transport [1]. Anemia is a reduction from the normal quantity of circulating hemoglobin in the blood less than 13 g/dL for male and less than 12 g/dL

for female adults [2]. Anemia effects are concentrated in preschool-aged children 40% and pregnant women 50%, making it a global public health problem [3, 4]. The availability of synthetic drugs used in the treatment of a specific disease is common but because of the high cost and side effects associated with their use [5], attention is currently being focused on the use of medicinal plant products in the prevention of human and animal health or management of various diseases or ailments [6]. Medicinal plants are used in various parts of the world especially in the tropics for the treatment of various forms of anemia [7]. Phenylhydrazine (PHZ) and its derivatives were first given a medical application at the end of the 19th century as antipyretics but due to its toxic action on red blood cells made their use dangerous [8]. Most anemia animal models are requiring a long time to induced anemia; thus, anemia is generally induced by cyclophosphamide, an anti-cancer agent, or by PHZ [9]. *Beta vulgaris* L. is crop belonging to the Chenopodiaceae family, popularly known by several common names like beetroot, table beet, garden beet, red beet, or golden beet and their leaves (called beet greens) [10]. Today, beetroot is grown in many countries worldwide, is regularly consumed as part of the normal diet [11]. Beetroot is one of the richest sources of fiber, vitamin C, folate, potassium, manganese, and iron [12]. Preliminary phytochemical and chromatographic studies found betacyanins and betaxanthins, flavonoids, polyphenols and vitamins in leaves [13]. Several parts of this plant are used in traditional Indian medicine for numerous therapeutic properties. Leaves are tonic, diuretic and useful in alleviating inflammation, paralysis, and diseases of spleen and liver [14, 15]. Due to the high nutritional value, leaves are widely consumed as vegetables worldwide. These phytochemicals have been reported as a potent antioxidant [16] and hepatoprotective

compounds [17]. The aim of this study was to determine the iron and vitamins contents in different parts of beetroot as root and beet green (leaves and stalk together) as well as their ethanol extracts. Then, selecting the best of them to evaluate it for reducing anemia caused by phenylhydrazine in rats.

2. MATERIALS AND METHODS

2.1. Chemicals

Phenylhydrazine was purchased from Sigma Chemical Co. (St Louis, MO, USA) and solvents were purchased from Merck (Darmstadt, Germany). Kits used for the estimation of analyzed parameters were purchased from BioSystems, Spain.

2.2. Plant material

The beetroot (*B. vulgaris* L.) was collected from the local market in Cairo, Egypt. Beetroot was identified and authenticated by Dr. Samah Azooz from the Botany Department, Faculty of Agriculture, Cairo University, Egypt.

2.3. Preparation of samples

Freshly collected plant material was washed thoroughly 2-3 times with distilled water then cut into two parts; the first part is root and the second part is beet green (leaf and stalk together). The samples were air dried in a ventilated oven at 40 °C for 48 h and ground to a fine powder. The dried samples powders were stored in polyethylene bags in the refrigerator at 4 °C until further use.

2.4. Preparation of extract

10 g of dried plant materials were extracted with 100 mL of 90% absolute ethanol at room temperature with constant shaking for 6 h. The extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The residues were re-extracted twice with the same fresh solvent and extracts combined. The combined extracts were evaporated under

reduced pressure at 45 °C using rotary evaporator (RV 10 digital, Germany). The two crude extracts were placed in dark bottles and stored in a freeze -4 °C until analyzing time [18].

2.5. Estimation of iron

The iron content of dried root and dried beet green of beetroot as well as their ethanolic extracts were analyzed using the methods described by Onwuka [19].

2.6. Estimation of vitamins

Hematopoietic vitamins such as riboflavin (vitamin B₂), pyridoxine (vitamin B₆), cyanocobalamin (vitamin B₁₂) and folic acid of dried root and dried beet green of beetroot as well as their ethanolic extracts were analyzed by reversed-phase HPLC using the methods described by Jin *et al.* [20].

2.7. Chemical composition and oxalate content of dried beet green

Moisture, ash, crude protein, the fat and dietary fiber of dried beet green were analyzed by the AOAC methods [21]. The carbohydrate content was calculated by the difference [22]. Oxalate content was determined according to the method described by AOAC [23].

2.8. Diet composition

Standard diet, vitamin and mineral mixtures were prepared according to AIN-93G (American Institute of Nutrition, 1993 G) [24]. Anti-anemic diet, which contained 5% dried beet green, has been modified by adjusting the various components of protein, fat, carbohydrates and fiber to be balanced with a control diet.

2.9. Animals

Wistar male albino rats weighing around (165 ±5 g) were purchased from the Animal Care Center, Faculty of Veterinary Medicine, Cairo University. The animals were housed in a separated cage at constant temperature (25 °C ± 2 °C) humidity (55%), and light-dark conditions (12/12 h light/dark ratio) and given a standard

diet and water ad libitum through the adaptation period (one week). The experiments were carried out in the animal house of the Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt accordance with the guide for the care and use of laboratory animals [25] and the protocol was approved by the Institutional Animal Ethics Committee.

2.10. Induction of experimental anemia

For induction of the model of hemolytic anemia, PHZ was dissolved in 0.9% NaCl and injected intraperitoneal at 40 mg/kg body mass for 2 days at 9 am and 6 pm as described previously [26]. Anemia was considered to be induced when red blood cell (RBC) level as well as a hemoglobin concentration of the blood reduced by about 30%.

2.11. Experimental protocol

The rats were randomly divided into four groups (n=10/group). Group, I fed a standard diet (normal control). Group II fed standard diet contained 5% dried beet green. Group III fed a standard diet with induction of anemia (anemic control) while group IV fed a standard diet containing 5% dried beet green with the induction of anemia. The body mass of animals was weighed at D3, D21 and D42 days. At the end of the feeding experimental period, the rats were fasted overnight before sacrificing and the organs (heart, kidney liver, and spleen) from each group were quickly removed, washed with ice-cold saline to remove blood, dried between filter papers and weighed to calculate the relative percentage of organs to body mass.

2.12. Collection of blood sample

Blood samples were taken from the retro-orbital vein after fasting of 12h using a glass capillary tube at D3, D21 and D42 days. Each sample was divided into three tubes, the first containing EDTA for hematological analysis. The second tube containing EDTA for

separation of plasma and the last tube used for separation of serum. The serum and plasma were separated by centrifugation (Hettich, Universal 16, German) at 560 g for 15 min at 4 °C, then collected into sterilized tubes and stored at -20 °C for further biochemical analysis.

2.13. Analyses of haematological parameters

RBC, WBC, Hb, and HCT were determined using Automated Hematology Analyzer (XT-2000i, Sysmex Corporation, KOBE, JAPAN).

2.14. Analyses of biochemical parameters

Serum AST, ALT, and ALP were determined using kits obtained from BioSystems S. A., Barcelona, Spain according to the method of Friedman and Young [27]. Also, serum creatinine, urea, and uric acid were determined using kits obtained from BioSystems S.A., Barcelona, Spain according to the method of Fabing and Ertingshausen, Tabacco *et al.* and Fossati *et al.* [28- 30]. Serum MDA, plasma H₂O₂ and TAC were analyzed using kits obtained from Biodiagnostics Co., Egypt according to the method of Onkawa *et al.*, Aebi and Koracevic *et al* [31-33]. Determinations were carried out according to the manufacturer's instructions.

2.14. Histopathological studies

The heart, kidney liver, and spleen from each group were fixed in 5% buffered formalin and embedded in paraffin wax. Microtome sections of 3-4 µm thickness were prepared according to the standard procedure and stained with hematoxylin and eosin (400×). Sections were then examined by the light microscope [34].

2.15. Statistical analysis

All data recorded were expressed as the mean ± standard deviation (SD). The data of the groups were statistically performed by variance

(ANOVA) procedures. A significant difference between groups was obtained by Duncan's Multiple Range Test (DMRT) SPSS software for Windows Version 20.0 (IBM Corp. Armonk, New York, NY, USA) at a level of P<0.05.

3. RESULTS AND DISCUSSION

3.1. Iron content

Iron is an essential trace element for hemoglobin formation. Iron content was higher in dried root and beet green compared to their ethanolic extracts. The dried beet green had high iron content (308.47 ± 1.31 mg/kg) followed by dried root (84.33 ± 0.57 mg/kg). While, iron contents of beetroot ethanolic extracts from the root and beet green were 2.83 ± 0.15 and 4.92 ± 0.16, respectively. Agree with, the green leafy powder of *beta vulgaris* had the highest iron content followed by green leafy powders of turnip (*Brassica rapa*) and carrot (*Daucus carota*), respectively [35].

3.2. Vitamins contents

Table 1 shows the contents of vitamins contents such as riboflavin (vitamin B₂), pyridoxine (vitamin B₆), cyanocobalamin (vitamin B₁₂) and folic acid of the dried beetroot from root and beet green as well as their ethanolic extracts. The dried beet green has been rich sources of vitamins B₆, B₁₂, and Folic acid; while the root ethanolic extract has been a rich source of vitamin B₂. The same result was reported that beetroot is one of the richest sources of iron, folate and a number of vitamins [12]. From this finding, it was found that green beet was a rich source of vitamins B₆, B₁₂, and folic acid so the chemical composition of this part was estimated to prepare a diet to reduce anemia caused by phenylhydrazine in a rat.

Table 1. Contents of vitamins (mg/100 g) in different parts of beetroot as well as their ethanolic extracts

Vitamins (mg/100 g)	Powder		Ethanolic extract	
	Root	Beet green	Root	Beet green
B ₂	4.88±0.12 ^c	10.76±0.17 ^b	58.82±0.19 ^a	4.22±0.09 ^d
B ₆	5.29±0.16 ^c	9.96±0.08 ^a	7.15±0.13 ^b	5.30±0.05 ^c
B ₁₂	52.77±0.14 ^c	279.41±1.29 ^a	40.81±0.11 ^d	222.27±2.12 ^b
Folic acid	7.06±0.10 ^d	19.71±0.32 ^a	10.96±0.25 ^c	15.26±0.24 ^b

Values are means ± SD. Within the same raw various superscript letters indicate significant differences by Duncan's test (p<0.05). B₂, vitamin riboflavin; B₆, vitamin pyridoxine; B₁₂, vitamin cyanocobalamin

3.3. Chemical composition and oxalate content of dried beet green

Moisture, ash, crude protein, crude fat, crude fiber and carbohydrate contents of the beet green were 5.62±0.05, 18.82± 0.26, 1.43± 0.02, 22.39± 0.14, 18.08± 0.21, and 33.66± 0.40 % dry matter, respectively. The oxalate content was 7.09±0.06 g/100 g dry matter and its content in the same range of commonly consumed leafy vegetables that are eaten raw, like spinach [36]. For this reason, 5% of dried beet green has been added to the standard diet, so the daily oxalate intake is about half of the recommended amount (152 ± 83 mg/day) [37].

3.4. Body mass and percentage mass gain

Table 2 shows the body mass; body mass and percentage mass gain of phenylhydrazine-induced anemic rats fed standard diet contained 5% dried beet green for 42 days. Results showed that there was non-significant difference in the initial masses of rats in all groups. However, there was a significant (p<0.05) decrease in the body mass in groups III and IV, respectively at the day D21. The anemia-induced group injected with PHZ showed a significant decrease in mass compared to that in the normal group (P<0.05) [38]. Mass loss in the PHZ-treated group suggested that nutrition deteriorated due to

oxidation losses in tissues from oxygen free radicals due to the automatic oxidation of PHZ, a strong oxidant [39]. Furthermore, feeding rats on standard diet contained 5% dried beet green with induction of anemia (group IV) reversed the mass loss (g) to the normal level (g) at the day D42. On the other hand, the highest significant (p<0.05) increase in the final mass was found in group II compared to group I at the D21 and D42 days. Results also show that group III has 1.57% mass gain compared with that of group I which was 26.98%, while groups II and IV have 32.90 and 26.91% mass gain, respectively.

3.5. Relative percentage of organs to body mass

Table 3 shows the relative percentage of organs to body mass (heart, kidney, liver, and spleen) of phenylhydrazine-induced anemic rats fed standard diet contained 5% dried beet green. At day D42, there were no significant (p<0.05) difference in the relative percentage of heart, kidney, liver, and spleen to body mass for all treated groups except group III showed a significant increase in the relative percentage of liver and spleen to body mass compared to control group. This indicates that the dried beet green was not toxic for their organs.

Table 2. Body mass (g) and percentage mass gain (%) of phenylhydrazine-induced anemic rats fed experimental diets

Groups	Body mass during the experiment (g)			Mass gain%
	D3	D21	D42	
I	165.33± 2.16 ^a	193.67± 3.98 ^b	226.50± 4.09 ^b	26.98± 1.85 ^b
II	166.50± 3.78 ^a	209.50± 3.45 ^a	248.17± 1.94 ^a	32.90± 1.75 ^a
III	166.67± 3.72 ^a	161.50± 4.18 ^d	169.33± 3.01 ^c	1.57± 2.00 ^c
IV	165.00± 3.74 ^a	186.33± 3.56 ^c	225.83± 4.71 ^b	26.91± 2.12 ^b

Values are means ± SD. Within the same column, various superscript letters indicate significant differences by Duncan's test ($p < 0.05$). Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anaemia (anaemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anaemia; D3, third day; D21, twenty-one day; D42, forty-two day.

Table 3. Relative percentage (%) of organs to body mass of phenylhydrazine-induced anemic rats fed experimental diets

Groups	The relative percentage of organs to body mass (%)			
	Heart	Kidney	Liver	Spleen
I	0.35±0.01 ^a	0.80±0.04 ^a	3.27±0.06 ^b	0.37±0.04 ^b
II	0.35±0.01 ^a	0.79±0.01 ^a	2.29±0.03 ^b	0.37±0.02 ^b
III	0.36±0.03 ^a	0.81±0.03 ^a	5.20±0.11 ^a	0.58±0.04 ^a
IV	0.36±0.02 ^a	0.79±0.03 ^a	3.29±0.05 ^b	0.39±0.04 ^b

Values are means ± SD. Within the same column, various superscript letters indicate significant differences by Duncan's test ($p < 0.05$). Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anemia (anemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anemia.

3.6. Hematological parameters

As shown in **Table 4 and 5**, there were significant decreases in RBC count, HGB concentration and HCT percentage, as well as the number of WBC, were significant increases after D3 day of PHZ injection compared with control group. Previous studies demonstrated that hematological data showed a significant

drop in HGB concentration, RBC count and HCT scores in rat's post-injection of PHZ as compared to the control group [40-42]. This could be as a result of the toxicity induced by phenylhydrazine, by peroxidation of RBC membrane lipids and this effect may be a result of the auto-oxidation of the drug and the interaction of oxygen radicals with membrane

lipids [43]. After fed standard diet containing 5% dried beet green with induction of anemia (group IV), we see an increase in RBC count, HGB concentration and HCT percentage while the number of WBC was improved at day D21. These hematological indices in group IV returned to normal ranges at day D42. Vitamins such as folic acid, vitamin B12, riboflavin, and vitamin B6, are necessary for the normal production of red blood cells [44]. The return hematological indices in group IV to normal ranges after six week may be due to the presence of iron, Vit. B₆, Vit. B₁₂ and folic acid in the beetroot leaf which could be reversed the effects of phenylhydrazine drug. These results are agreements with vitamin and minerals found in beetroot are most likely active ingredients responsible for its hematinic effects [45]. On the other hand, the highest significant increases of RBC count, HGB concentration and HCT percentage showed in group II compared with control group at D21 and D42 days. The previous study showed after 20 days of taking 8 g of beetroot on a daily basis of female volunteers the levels of total iron binding capacity (TIBC) decreased and serum iron levels increased. This indicates that beetroot is an excellent source of iron. In addition, the last result found a mild increase in HGB concentration and thus it can be stated that beetroot might have some therapeutic properties for iron deficiency [46].

3.7. Serum hepatic enzymes

As shown in **Fig. 1-3** a significant elevation in the activities of serum liver enzyme markers AST, ALT and ALP were observed in III and IV groups compared to control group I at day D3. This observation is in agreement with previous studies founding that there were significant increases in the liver biomarker enzymes of the anemic group when compared to the normal

control group. This may be due to the toxicity induced by phenylhydrazine [47, 48]. At day D21, the activities of these enzymes were significantly lower in group IV compared to group III. On the other hand, no significant ($p < 0.05$) difference were observed in the enzyme activities of ALT and ALP between group IV and group I at day D42. While at the same day a slight increase in AST enzyme activity was observed in group IV compared to group I. Previous phytochemical studies have shown the presence of betalains, flavonoids, phenolics, vitamins, and minerals and in *beta vulgaris* Linn leaves [13]. These phytochemicals might be responsible for the hepatoprotective effect against ethanol-induced hepatic toxicity [17]. Our findings also revealed that the activities of serum liver enzyme markers did not change in group II compared to the control group at days D21 and D42. The non-significant increase of these enzymes may imply that the fed standard diet contained 5% dried beet green is safe and non-toxic to the liver.

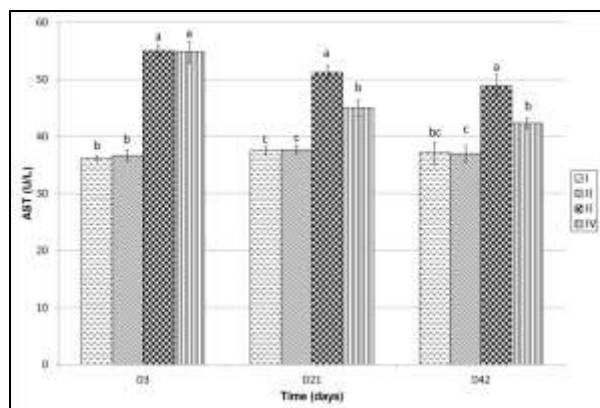


Fig. 1. Serum AST enzyme activity (U/L) of phenylhydrazine-induced anemic rats fed experimental diets. Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anaemia (anaemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anaemia; D3, third day; D21, twenty-one day; D42, forty-two day; AST, aspartate aminotransferase.

Table 4. RBC counts ($10^6 \mu\text{L}$) and HGB concentration HGB (g/dL) of phenylhydrazine-induced anemic rats fed experimental diets

Groups	Hematological parameters					
	RBCs ($10^6 \mu\text{L}$)			Hb (g/dL)		
	D3	D21	D42	D3	D21	D42
I	6.75±0.58 ^a	6.96±0.31 ^b	7.05±0.29 ^b	13.19±0.62 ^a	13.50±0.72 ^b	14.17±0.48 ^b
II	6.73±0.55 ^a	7.50±0.57 ^a	8.08±0.48 ^a	13.03±0.52 ^a	15.00±0.84 ^a	17.06±0.54 ^a
III	3.83±0.40 ^b	3.90±0.32 ^d	3.92±0.32 ^c	7.99±0.54 ^b	8.83±0.62 ^d	9.33±0.57 ^c
IV	3.84±0.41 ^b	5.14±0.50 ^c	7.02±0.24 ^b	8.01±0.29 ^b	11.49±0.58 ^c	14.33±0.58 ^b

Values are means ± SD. Within the same column, various superscript letters indicate significant differences by Duncan's test ($p < 0.05$). Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anemia (anemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anemia; D3, third day; D21, twenty-one day; D42, forty-two day; RBC, red blood cell; Hb, hemoglobin.

Table 5. HCT percentage (%) and WBC counts ($10^3 \mu\text{L}$) of phenylhydrazine-induced anemic rats fed experimental diets

Groups	Hematological parameters					
	HCT (%)			WBC ($10^3 \mu\text{L}$)		
	D3	D21	D42	D3	D21	D42
I	37.72±2.42 ^a	38.53±1.13 ^b	39.37±0.94 ^b	15.86±2.01 ^b	16.93±1.60 ^c	18.49±1.92 ^b
II	37.62±2.28 ^a	41.87±1.43 ^a	44.52±0.94 ^a	16.02±2.15 ^b	17.59±1.97 ^c	18.25±1.66 ^b
III	28.24±1.03 ^b	29.04±1.35 ^d	31.71±1.41 ^c	32.03±1.21 ^a	30.29±1.66 ^a	27.79±1.89 ^a
IV	28.91±1.15 ^b	34.24±1.03 ^c	39.06±1.00 ^b	32.12±3.16 ^a	25.75±1.83 ^b	18.75±1.07 ^b

Values are means ± SD. Within the same column, various superscript letters indicate significant differences by Duncan's test ($p < 0.05$). Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anemia (anemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anemia; D3, third day; D21, twenty-one day; D42, forty-two day; HCT, haematocrit; WBC, white blood cell.

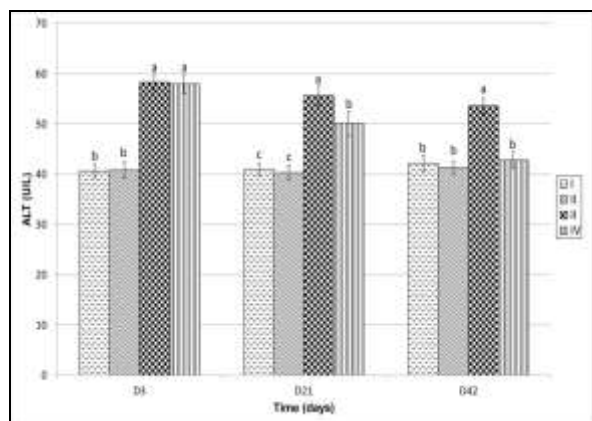


Fig. 2. Serum ALT enzyme activity (U/L) of phenylhydrazine-induced anemic rats fed experimental diets. Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anemia (anemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anemia; D3, third day; D21, twenty-one day; D42, forty-two day; ALT, alanine aminotransferase.

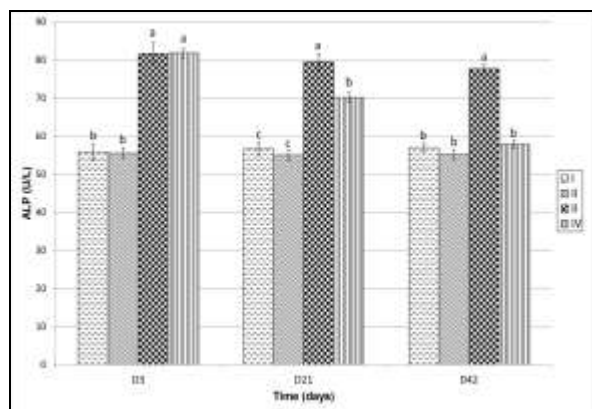


Fig. 3. Serum ALP enzyme activity (U/L) of phenylhydrazine-induced anemic rats fed experimental diets. Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anaemia (anaemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anaemia; D3, third day; D21, twenty-one day; D42, forty-two day; ALP, alkaline phosphatase.

3.8. Serum renal parameters

The results are presented in **Fig. 4-5** showed that the level of serum creatinine and urea increased significantly in group III compared to

group I at days D3, D21, and D42. On the other hand, group IV which feeding 5 % dried beet green showed that the levels of serum urea and creatinine increased significantly at day D3 while they decreased at day D21 and returns them to normal levels at D42 day compared to control group. The same results were found from previous studies which showed that renal dysfunction and structural damage have been attenuated using beetroot through the reduction of oxidative stress, inflammation, and apoptosis in the kidney [49]. While, group II did not show any significant change in the levels of these renal parameters as compared to group I at days D3, D21, and D42. In addition, a non-significant difference was observed in the level of serum uric acid in all treated groups compared to group I. Thus, the result of the present study revealed that the dried beet green is non-toxic to the kidney.

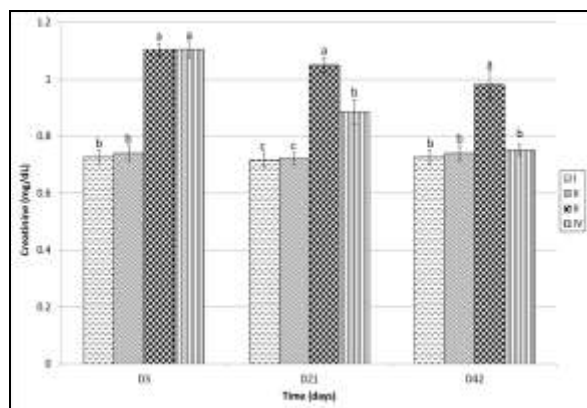


Fig. 4. Serum creatinine level (mg/dL) of phenylhydrazine-induced anemic rats fed experimental diets. Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anaemia (anaemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anaemia; D3, third day; D21, twenty-one day; D42, forty-two day.

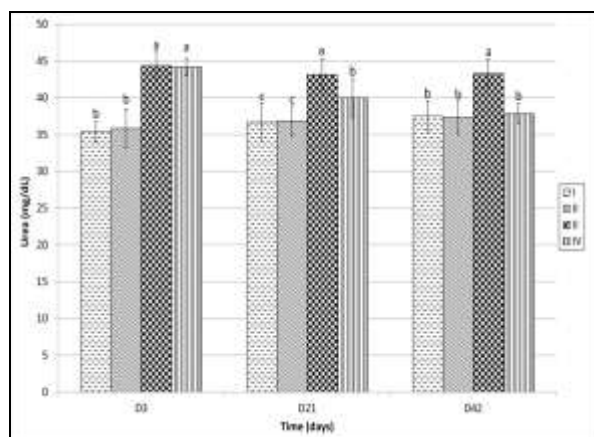


Fig. 5. Serum urea level (mg/dL) of phenylhydrazine-induced anemic rats fed experimental diets. Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anaemia (anaemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anaemia; D3, third day; D21, twenty-one day; D42, forty-two day

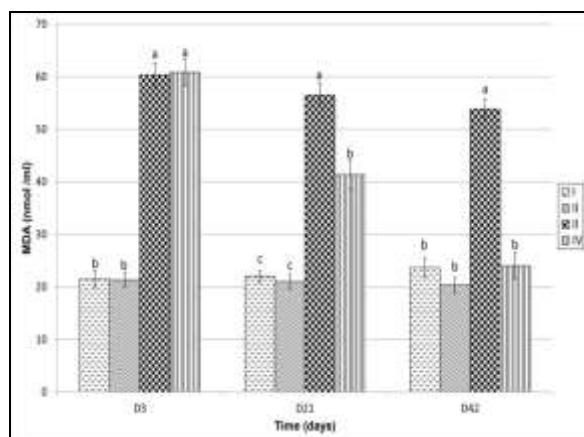


Fig. 6. Serum MDA (nmol/mL) level of phenylhydrazine-induced anemic rats fed experimental diets. Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anaemia (anaemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anaemia; D3, third day; D21, twenty-one day; D42, forty-two day; MDA, malondialdehyde.

3.9. Serum TBARS, plasma H₂O₂, and TAC levels

As demonstrated in **Fig 6-8**, injection of PHZ into rats had associated with a marked elevation in serum MDA and plasma H₂O₂ levels as well as significant reduction in the level of TAC. These results suggesting that it may have caused oxidative stress. Previous studies demonstrated that PHZ treatment was followed by an elevation in the levels of plasma TBARS [50] and H₂O₂ [51]. In group IV, feeding 5% dried beet green reduced serum MDA and plasma H₂O₂ levels as well as improved the level of TAC at D21 day and returns them to normal levels at D42 day. The serum MDA, plasma H₂O₂, and TAC levels were not different in group II compared to the control group at days D21 and D42.

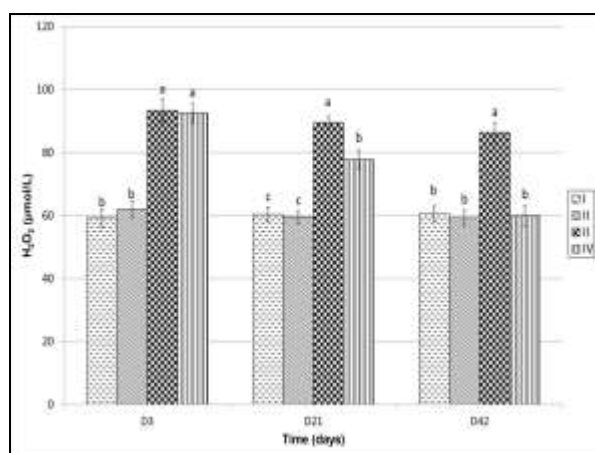


Fig. 7. Plasma H₂O₂ (µmol/L) level of phenylhydrazine-induced anemic rats fed experimental diets. Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anemia (anemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anemia; D3, third day; D21, twenty-one day; D42, forty-two day; H₂O₂, hydrogen peroxide.

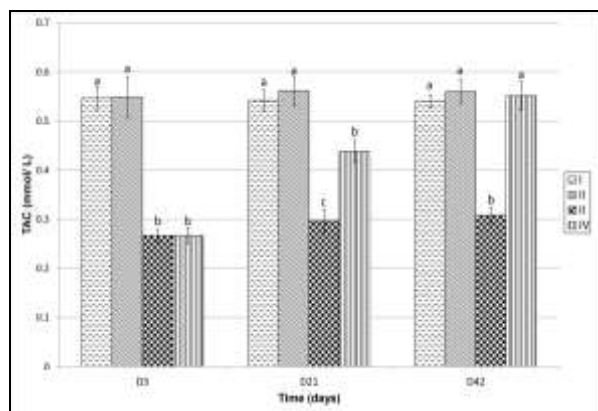


Fig. 8. Plasma TAC (mmol/L) level of phenylhydrazine-induced anemic rats fed experimental diets. Group I, fed standard diet (normal control); Group II, fed standard diet contained 5% dried beet green; Group III, fed standard diet with induction of anaemia (anaemic control); Group IV, fed standard diet contained 5% dried beet green with induction of anaemia; D3, third day; D21, twenty-one day; D42, forty-two day; TCA, total antioxidants capacity.

3.10. Histological examination

3.10.1. Heart

Fig. 9 illustrates the effect of feeding 5% dried beet green on histopathology examinations of heart tissues in phenylhydrazine-induced anemic rats. Heart of rats from the group I showed normal cardiac myocytes (Fig. 9a). Group II rats fed standard diet contained 5% dried beet green showed no histopathological changes (Fig. 9b). Meanwhile, the heart of rats from group III revealed focal necrosis of cardiac myocytes associated (Fig. 10c) and intramuscular inflammatory cells infiltration (Fig. 9d). However, the heart of rats from group IV showed no histopathological changes (Fig. 9e).

3.10.2. Kidney

Fig. 10 illustrates the effect of feeding 5% dried beet green on histopathology examinations of kidney tissues in phenylhydrazine-induced anemic rats. Kidney of rats in groups I, II and IV showed the normal histological structure of renal tubules (Fig. 10 a, b, and d, respectively).

Meanwhile, kidney of rats from group III revealed vacuolization of epithelial lining renal tubules as well as vacuolation and congestion of endothelial lining glomerular tuft (Fig. 10c). Previous studies have reported that the kidney section obtained from a rat treated with phenylhydrazine showed histological changes which include degeneration of the glomerulus and focal diffuse tubular degeneration and lymphocytic infiltration [52].

3.10.3. Liver

Fig. 11 illustrates the effect of feeding 5% dried beet green on histopathology examinations of liver tissues in phenylhydrazine-induced anemic rats. A normal histological structure of hepatic lobule was observed in the liver tissues of the control rats (Fig. 11a). Group II rats fed standard diet contained 5% dried beet green showed no histology change of the liver similar to the control group (Fig. 11b). However, very degenerative changes in the liver tissue of the anemic rats were seen including sporadic necrosis of hepatocytes, Kupffer cells activation (Fig. 11c), cytoplasmic vacuolation of hepatocytes (Fig. 11d), cystic dilatation of bile duct and fibroplasia around the bile duct (Fig. 11e). On the other hand, the liver of rats from group IV showed no histopathological changes (Fig.11f) and few examined sections revealed Kupffer cells activation (Figs. 11g).

3.10.4. Spleen

Fig. 12 illustrates the effect of feeding 5% dried beet green on histopathology examinations of spleen tissues in phenylhydrazine-induced anemic rats. A normal histological structure of lymphoid follicle was observed in the spleen tissues of the control rats (Fig. 12a). Group II rats fed standard diet contained 5% dried beet green showed no histology change of the spleen similar to the control group (Fig. 12b). However, very degenerative changes in the spleen tissue of the

anemic rats were seen including lymphocytic necrosis, apoptosis, and depletion (Fig. 12c). On the other hand, spleen of rats from group IV

showed no histopathological changes (Fig. 13d) whereas, few examined sections revealed slight lymphocytic depletion (Figs. 12e).

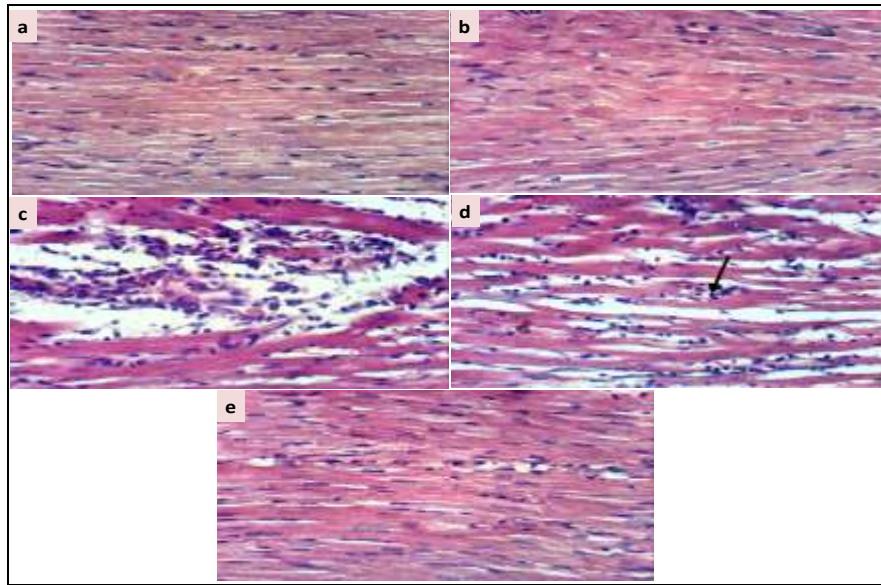


Fig. 9. Histopathological changes in the hearts of phenylhydrazine-induced anemic rats fed experimental diets (H and E, 400 \times). a: fed standard diet (normal control), b: fed standard diet contained 5% dried beet green, c-d: fed standard diet with induction of anemia (anemic control) and e: fed standard diet contained 5% dried beet green with induction of anemia.

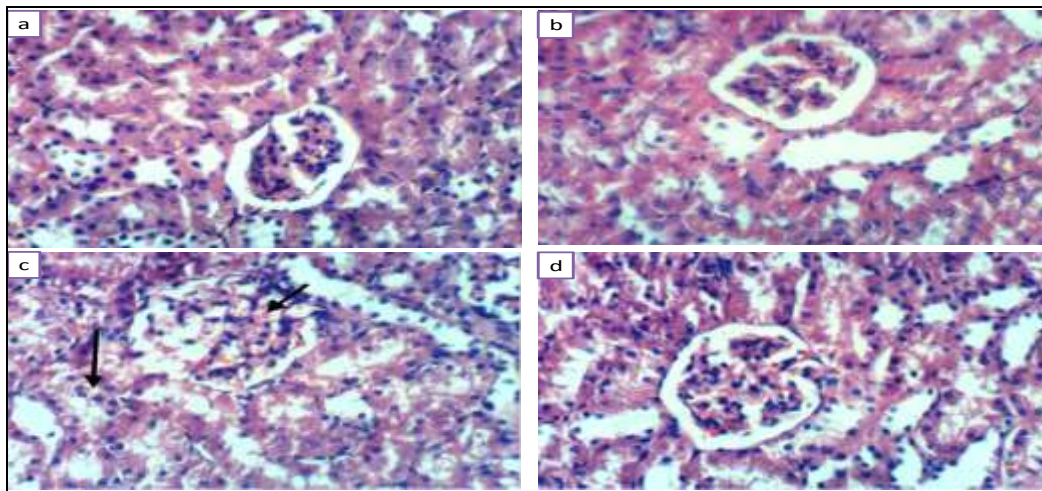


Fig. 10. Histopathological changes in kidneys of phenylhydrazine-induced anemic rats fed experimental diets (H and E, 400 \times). a: fed standard diet (normal control), b: fed standard diet contained 5% dried beet green, c: fed standard diet with induction of anemia (anemic control) and d: fed standard diet contained 5% beet green with induction of anemia.

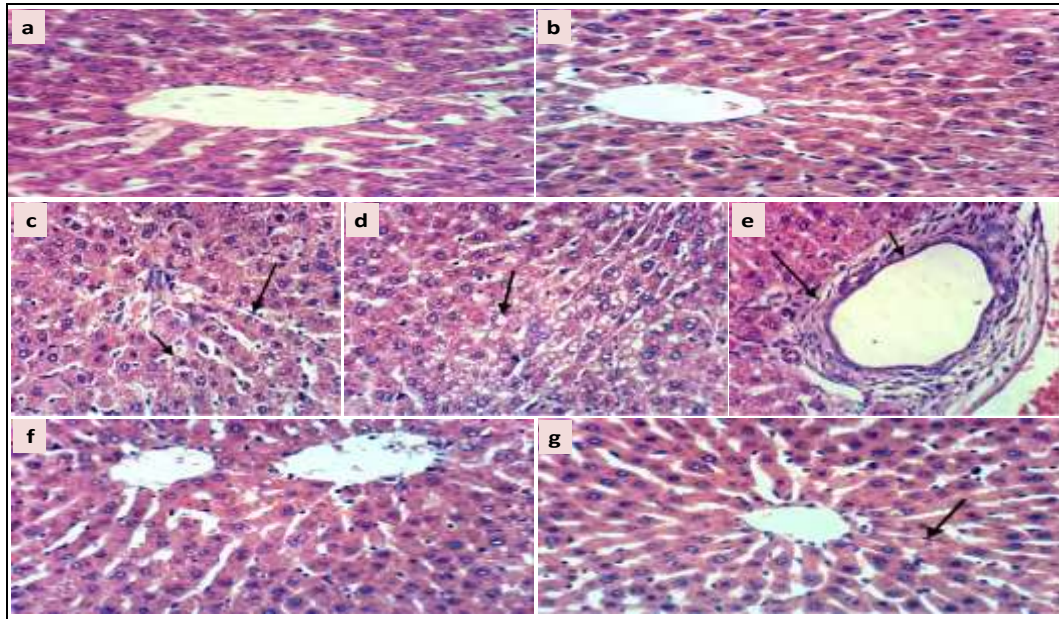


Fig.11. Histopathological changes in livers of phenylhydrazine-induced anemic rats fed experimental diets (H and E, 400 \times). a: fed standard diet (normal control), b: fed standard diet contained 5% dried beet green, c-e: fed standard diet with induction of anemia (anemic control) and f-g: fed standard diet contained 5% dried beet green with induction of anemia.

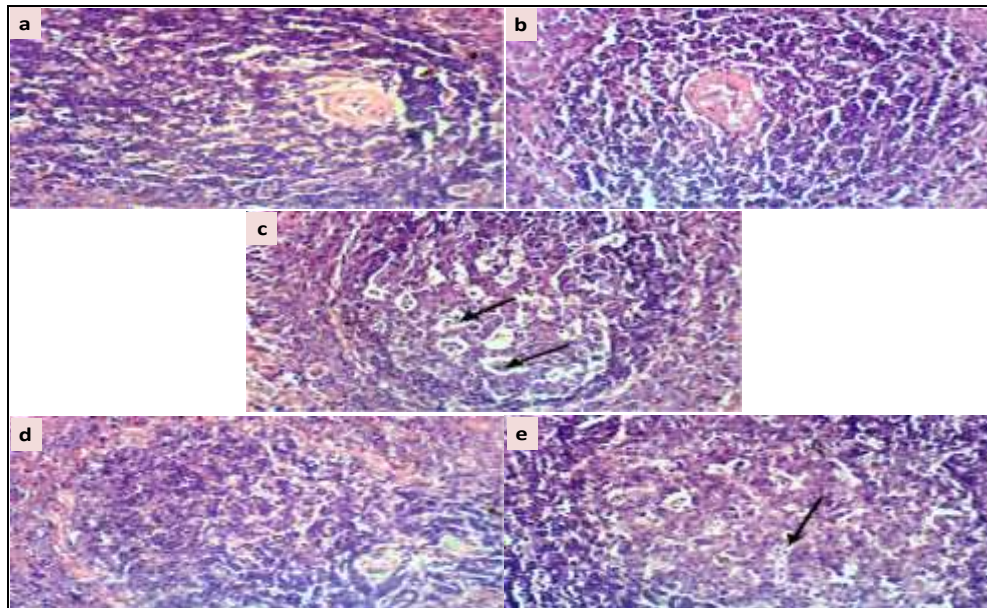


Fig. 12. Histopathological changes in spleens of phenylhydrazine-induced anemic rats fed experimental diets (H and E, 400 \times). a: fed standard diet (normal control), b: fed standard diet contained 5% dried beet green, c: fed standard diet with induction of anemia (anemic control) and d-e: fed standard diet contained 5% dried beet green with induction of anemia.

4. CONCLUSION

The dried beet green exhibits anti-anemic activity against phenylhydrazine anemic induction for rats. The anti-anemic effect produced by the beetroot leaf powder may due to its high contents of iron, folic acid, and vitamins B₆ and B₁₂ in this part of the plant. Although the results of the current study revealed that fed 5% of green beet is nontoxic for the kidney, but recommendations should be taken to reduce the intake of people who have a tendency to form kidney stones.

Authors' contributions:

This work was carried out in collaboration between all authors. Shahenda, M. Elaby: designed the study, managed the biochemical parameters analysis and wrote the manuscript. Jehan, B. Ali designed the collected plant material literature searches and revised the written manuscript.

Disclosure

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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