Using Some Beetroot Products to Improve Some Parameters in Anemic Rats Induced by Phenylhydrazine

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ABSTRACT

Background: Anemia is a widespread disease among different countries. Anemia is caused by reducing erythrocyte growth, which leads to the advent of a large amount of underdeveloped erythrocytes. *Beta vulgaris* or beetroot is one of the important vegetables eaten worldwide and it used as traditional natural coloring agent in many cuisines. Medicinally, the beetroot has been used as a traditional medicine to prevent various diseases including anemia, liver, atherosclerosis, kidney and coronary heart disease.

objective: The aim of the present work was to evaluate anti-anemic potential of beetroot soup, salad and pudding in phenyl hydrazine-induced anemic rats.

Materials and methods: The chemical composition and antioxidant activity of beetroot were estimated. Various products have been prepared from beetroot, including salad, pudding and soup, and used for feeding healthy and anemic rats for 30 days to know the effect of these products on some important bioactive parameters.

Results: The results showed that beetroot contain moisture, protein, fat, ash, crude fiber, n-free extract and calories by 84.57±0.84, 13.02±0.56, 0.16±0.04, 15.49±0.21, 3.65±0.81, 67.68±0.02 and 324.24±0.55%), respectively. It was also found that beetroot are rich in phenolic compounds, flavonoids and antioxidant activity. The values revealed that beetroot products caused an improvement in the level of red blood cells, hemoglobin, MCV, MCH and MCHC in both (healthy and anemic) rats. Additionally, feeding healthy and anemic rats with beetroot products caused a significant increase in plasma total protein, albumin and globulin compared to PHZ group. Interestingly, the feeding with beetroot soup, salad and pudding restored the enzymes activities in plasma of healthy and anemic rats near to their normal levels. Moreover, beetroot products minimized the harmful effect of PHZ on the levels of plasma lipid profile and TBARS.

Finally, the present work recommended consumption of beetroot products for patients with anemia to improve hemoglobin levels, blood lipids, antioxidant enzymes, and the functions of both liver and kidneys.

Keywords: Anemia, rats ,Beetroot, phenylhydrazine, antioxidants.

INTRODUCTION

Anemia is a prevalent and public health issue in many developing countries, including Egypt and Saudi Arabia. Global estimates showed that 33% of non pregnant females and 43% of kids were anemic, with the greatest prevalence in South Asia and Africa **(Stevens *et al.*, 2013).** Regardless of the classifications and causes of anemia, it can be described as a situation with less than ordinary hemoglobin and/or red blood cells (RBC) count.

Such low concentrations of hemoglobin and RBCs may reduce the capacity of the blood to distribute oxygen to distinct body organs, leading to severe or life threatening uncontrolled anemia (**Gabrilove, 2000**). Among different anemias, hemolytic anemia is a prevalent type of anemia that may be inherited (owing to deficiency of glucose-6- phosphate dehydrogenase) or obtained owing to exposure to hemolytic agents) as a consequence of intra-(or extra vascular destruction of RBCs **(Silberstein and Anastasi, 2013).** Exposure to certain chemicals, including drugs, may be correlated with RBC destruction during treatment **(Beutler, 2001).**

Oxidative stress is also a direct cause and a predisposing factor of multiple blood disorders through the peroxidation of erythrocytic membranes **(Fibach and Rachmilewitz, 2008).** The innovation and discovery of therapeutic agents from secure sources received significant attention from pharmacologists owing to the key role that herbal medicine could play in disease prophylaxis and/or therapy, as well as enhancing the health status and efficiency of ordinary topics **(Mills and Bone, 2000).** In such a situation, proper dietary supplementation may be helpful.

Phenylhydrazine (Hydrazinobenzene) is the chemical compound characterized by Hermann Emil Fischer in 1895. It is mainly used as a chemical intermediate in the agrochemical, pharmaceutical, and chemical industries. PHZ derivatives were mainly used as antipyretics, but their use was hazardous owing to their poisonous action on red blood cells. Phenylhydrazine was mainly used for experimental induction of anemia in animals **(Pandey *et al.*, 2014).**

Several crops are used worldwide as food and for medicinal purpose. Medicinal plants have reported excellent potential in managing and treating multiple health problems. An example of such plants is the beetroot. Red beetroot is a vegetable feature of the Eastern and Central European diet and is additionally used as a typical folks remedy for urinary organ and liver diseases, to stimulate the hematopoietic and immune systems and as a unique diet in cancer treatment **(Kapadia *et al.*, 2003)**.

These beetroot medicinal properties are ascribed to their phytochemical and mineral structure. Beta carotene, tannins, saponins, alkaloids, phenols, coumarins, fatty acids, flavonoids, anthocyanins,, amino acids, triterpenes, and vitamins K, C, E and A have been identified in beetroot along with minerals such as calcium, copper, magnesium, iron, folic acid, manganese and potassium **(Chawla *et al.*, 2016).** In addition to other active chemicals, beetroot comprise a unique class of nonphenolic antioxidants, water-soluble, the betalains, including two groups of compounds, yellow betaxanthines and red betacyanins (mainlybetanin). Betalains' antioxidant impacts have been shown primarily in different in*vitro* studies **(Kanner *et al.*, 2001).** Today, beetroot is cultivated in many nations around the world, is frequently eaten as part of a standard diet and is widely used as a food coloring component known as E162 **(Georgiev *et al.*, 2010).**

MATERIALS AND METHODS

About 8 kg of fresh beetroot (*Beta vulgaris*) were obtained from the local market of vegetables and fruits, Alexandria Governorate, Egypt. Other materials used to prepare products were purchased from Alexandria market, Egypt. All (kits, chemicals and reagents) used in the study were purchased from El- Gomhoria Company, Alexandria, Egypt. Phenylhydrazine and DPPH– (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich Chemical Co. (USA). Preparation of beetroot products were carried out in the laboratories of Food Science and Technology Department, Faculty of Agriculture and Home Economics Department, Faculty of Specific Education, Alexandria University.

Preparation of beetroot products:

Beetroot was cleaned with tap water and peeled. The pulp was cut into small pieces " cubes" (1.5 × 1.5 × 1.5) cm, some portion was retained raw while other were used to prepare different products (juice, soup, pudding and salad).

Preparation of boiled juice

The beetroot cubes were boiled for 3,5 and 8 min in water (100° C). The ratio of the pulp to water was (1:1) (w/w). The mixture was blended in a mixer at a maximum velocity for 7 min. The juice was prepared to sensory evaluation test along with the control and then the juice was kept in polyethylene bags and frozen at - 18°C.

Preparation of beetroot soup

The soup was prepared by using beetroot juice, which was prepared by boiling beetroot for 5 min, this period was chosen because it gave the best result when conducting the sensory evaluation of the juice. While, traditional soup was prepared by boiling beetroot for 35 min.

The ingredients of soup are shown in **Table (1)**. The chopped raw onion, garlic, were fried in corn oil until these ingredients have golden color, then the other ingredients were added, cooked for 8 min and mixed with juice to make soup recipe.

Table (1): Ingredients of beetroot soup

Ingredient	Quantity (gm)
juice of beetroot	700
Chopped raw onion	70.6
Cloves	0.17
Chopped raw garlic	2.8
Black papper	0.23
Corn oil	7
Cumin	0.1
Food salt	6.5

Preparation of beetroot pudding

The ingredients of the pudding are summarized in **Table (2)**similar to **Saba (2005)**.The water, sugar, corn starch and beetroot juice were used for preparing pudding. The water was replaced by beetroot juice at three levels (25%, 50% and 75%). The control sample was prepared without any beetroot juice. Milk is not used in the preparation of pudding because milk prevents the absorption of iron. After weighing all components individually, they were all mixed together, and heated to 80–85 °C in a stainless-steel boiler for about 15 min, after that they were put in individual cups and cooled in the refrigerator.

Table (2): Ingredients of beetroot pudding

Ingredients (g)	Control sample (g)	Experimental samples%		
		25%	50%	75%
Beetroot juice	0	72	120	168
Water	240	168	120	72
Sugar	40	40	40	40
corn starch	10	10	10	10
Total	290	290	290	290

Preparation of beetroot salad

The ingredients of salad are shown in **Table (3)** according to **Saba(2005)**. The salad was prepared by boiling beetroot for 35 and 15min. The cubes of beetroot mixed with other ingredients to prepare salad.

Table (3): Ingredients of beetroot salad

Ingredient	Quantity (gm)
Boiled beetroot for 35 min (control)	700
Boiled beetroot for 15 min (treatment)	700
Corn oil	7
Cumin	0.1
Food salt	6.5

Organoleptic evaluation

The beetroot products was submitted to 20 panelists from staff members, students and employees of Faculty of Specific Education and Agriculture, Alexandria University for evaluation. The ranking method was used in combination with scoring based on the hedonic scale with 9 scores (1 = dislike extremely but 9 = like extremely) according to the method of (**Hood and Jood, 2005**).

Chemical analysis:-

Determination of chemical composition

Moisture, fat, ash and protein content were determined according to **AOAC(2007)**. The N-free extract content was obtained by subtracting the percent total of the fat, fiber, protein and ash contents from 100%.

Caloric values was calculated from the sum of the percentages of crude protein and N-free extract multiplied by factor of 4(kcal.g-1) plus the crude fat content multiplied by a factor of 9 (kcal.g-1) according to **Zambrano *et al.* (2004).** Minerals: Zn, Cu, Ca, Mn and Fe were determined using Atomic Absorption Spectrophotometer (Shimadzu model AA- 6650) as described in **AOAC (2000)**. Determination of vitamin C content according to **AOAC (2005)**.

Determination of total polyphenol , total flavonoids and antioxidant activities (DPPH) according to **Elfalleh *et al*. (2009), Čanadanovic´-Brunet *et al.* (2011)** and **Ravichandran *et al.*(2012)** respectively**.**

IC_{50} is described as the concentration of antioxidant needed to reduce the initial concentration of DPPH by 50%. The IC_{50} of the samples was obtained from the percentage of scavenging activity vs. concentration plot and is expressed as mg/ml.

Animal experiments

Eighty white male albino rats (Sprague dawley strain), weighing between (230±20g) were purchased from High Institute of Graduate Studies and Research, Alexandria University, Egypt. They were kept under observation for 5 days before experiment and fed on the basel diet and water *ad libitum* and kept for two weeks for acclimatization. The basel diet contains protein (13%) , choline (0.2%) , fat (4%) , vitamin mixture (1%) , cellulose (5%), salt mixture (3.5%) and the remainder was starch according to **Reeves *et al*.(1993**). The vitamin and the salt mixture were prepared according to **AOAC (1975) and Hegested *et al.* (1941)** respectively**.** The RBC number and hemoglobin concentration were determined before the rats were induced with anemia.

Grouping of rats:

After the incubation period, the rats were randomly classified into 8 groups (10 rats each). The first four groups were healthy rats, while the other four groups were injected with phenyl hydrazine to induced anemia. Anemia was induced by intraperitoneal injection of phenyl hydrazine (60 mg/kg, i.p., in divided doses daily, for 3 consecutive days) according to **Koffuor *et al.* (2011**). Anemia was considered to be induced when red blood cell (RBC) level as well as hemoglobin concentration in the blood reduced by about 30%. The control groups (negative (-ve) and (positive (+ve)) fed with basal diet only, while the other groups fed with beetroot salad, beetroot pudding and beetroot soup by 4g/kg B.W/ daily by stomach tube for 30 days. The ratios of the products given to the rats were determined according to a pre-test where most of the research deals with the extract or the powder. Therefore, in this research a pre-test was conducted to reach the best proportion of products led to the improvement of hemoglobin was applied in the experiment. Rats were given the products by oral gavage. The salads and pudding were carefully blended and diluted with a little of distilled water to facilitate swallowing by mouth.

During the experiment the RBC number and concentration of hemoglobin were determined every week in order to follow the level of anemia throughout the trial period. At the end of the experiment (4 weeks), rats were fasted overnight, then sacrificed under ether anesthesia, then blood samples were collected from the aorta. The blood samples were centrifuged and plasma was separated to determine some biochemical analysis.

Biochemical analysis:

Determination of haematological parameters

Main parameters of the red blood cell count evaluated using the methodology outlined by **Angelov *et al.* (1999)**. The following parameters were determined: - Number of red blood cell (RBC)– through the chamber method - with Burker's camera. Total white blood cell (WBC) counts were obtained using a Coulter counter in a well-standardized commercial laboratory. Hemoglobin content– by means of the cyan-hemoglobin method - at a wavelength of 540nm. Hematocrit - centrifugation in capillary tubes. Centrifugation was performed at 125 rpm for 5 min. Based on the values of the above parameters, we also calculate the mean corpuscular volume (MCV), the mean hemoglobin content (MCH) and the mean hemoglobin concentration in the cell (MCHC) as defined by the formulas of **Penev and Dukova-Peneva (2007).**

Determination of glucose, total protein and albumin

blood was heparinized for estimation of glucose, total protein (TP) and albumin were determined according to **Weissman and Klein (1958)**, **Armstrong and Carr (1964)** and **Doumas *et al.* (1971)** respectively**.** Globulin content was calculated by subtraction according to **Price *et al.* (1976)**.

Plasma globulin contents (g/dL) = blood plasma total proteins – blood plasma albumin.

Determination of lipid profile:

Total lipids (TL), triglycerides (TG), total cholesterol and high density lipoprotein cholesterol (HDL-C) were determined as outlined by **Frings *et al.* (1972)**, **Bucolo and David (1973)** and **Richmond (1973),** respectively**.** Low-density lipoprotein (LDL) was determined by the calculation (cholesterol-(TG/5+HDL).Very low-density lipoprotein (VLDL) was calculated by dividing the values of TG by factor of 5. Athero genic index (AI) was determined by the calculation

$$\frac{\text{LDL+VLDL}}{\text{HDL}}$$

Determination of enzymatic antioxidants activity:

glutathione (GSH**),** glutathione-S-transferase (GST), glutathione Peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and the thiobarbituric acid reactive test (TBARS) were evaluated using the method of **Ellman (1959**), **Habig *et al*. (1974), Paglia and Valentine (1967), Aebi (1984), Mishra and Fridovich (1972)** and **Tappel and Zalkin (1959),** respectively**.** All this enzymes were determined by Bio-diagnostic Kit, Egypt.

Determination of liver and kidney functions

Urea, creatinine, uric acid, aspartate aminotransferase (AST) and alanine amino transferase (ALT) activities, alkaline phosphatase (AlP) and acid phosphatase (ACP) were measured according to **Patton and Crouch (1977)**, **Schirmeister (1964), Barham and Trinder (1972), Reitman and Frankel (1957), Belfield and Goldberg (1971)** and **Daniel *et al.* (1954),** respectively**.**

Statistical analysis of the data

Data was transmitted to the computer and analyzed using IBM SPSS software package version 20.0**. (**Armonk, NY: IBM Corp**) (Kirkpatrick and Feeney, 2013)**.Quantitative data were described using mean, standard deviation. Significance of the obtained results was judged at the 5% level **(Kotz *et al.* ,2006) .**The used tests were F-test (ANOVA) For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (LSD) for pair wise comparisons.

RESULTS AND DISCUSSION

Chemical composition

The proximate composition of raw beetroot showed in **Table (4-a)**.The values of moisture, protein, fat, ash, crude fiber, n-free extract and calories were found to be 84.57±0.84, 13.02±0.56, 0.16±0.04, 15.49±0.21, 3.65±0.81, 67.68±0.02% and 324.24±0.55 kal/100g) respectively. **Vulić *et al.* (2012)** found that the edible roots of red beet contain from12to 20 % dry matter, including 1.5 % protein, 4 –12 % sugar, 0.8 % fiber, 0.1 % fat. Also**, Kale *et al.* (2018)** found that the moisture content was 87.4%, fat 0.3%, protein 1.35%, carbohydrates 7.59%, crude fiber 1.9%, and ash 1.4%. This results found to be similar with **Odoh and Okoro (2013)**. In this respect **Guldiken *et al.*(2016)** found that the highest moisture contents was measured for red beetroot juice (93 %) and pickled red beetroot (92 %) samples; red beetroot jam had the smallest moisture content value (17 %).

Table (4-a):Proximate chemical composition of beetroot powder (DW%)

Parameters	Value %
Moisture (%)	84.57±0.84
Protein (%)	13.02±0.56
Crude Fats (%)	0.16±0.04
Ash(%)	15.49±0.21
Crude Fibers (%)	3.65±0.81
N-free Extract(%)*	67.68±0.02
Calories (kal/100g)	324.24±0.55

Values represent means ± standard deviation of triplicates.
* N-free Extract(%) calculated by difference.

The minerals content of raw beetroot (on dry weight basis) showed in Table (4-b). The values of Ca, Mn, Zn, Cu and Fe were found to be 48.47±0.88, 17.04±0.22, 13.73±0.30, 10.69±0.63 and 407.84±0.64 mg/100g, respectively. It is clear that the beet contains a large amount of iron. **Vulić *et al.* (2012)** found that the edible roots of red beet contain several minerals such as calcium, sodium, iron, phosphorus, and potassium, but also a small amounts of vitamins. The mineral composition of beetroot were analyzed and results revealed that iron was 0.76, potassium 30.12, zinc 4.9, sodium 73.60 and copper 0.08 (mg/100g), respectively **(Kale *et al.*, 2018)**.

Also, **Ingle *et al.* (2017)** reported that with enhanced levels of beetroot powder in cookies, the calcium, phosphorous and iron content of cookies improved with decreased zinc content and cookie calorific value. Results showed that greater amounts of beetroot powder replaced by formulation resulted in enhanced mineral content in cookies**.**

Table (4-b):The minerals content of raw beetroot (on dry weight basis)

Minerals	Value(mg/100g)
Ca	48.47±0.88
Mn	17.04±0.22
Zn	13.73±0.30
Cu	10.69±0.63
Fe	407.84±0.64

Values represent means ± standard deviation of triplicates.

Bioactive components and antioxidant activity of beetroot

The values of total phenols, total flavonoids and vitamin C in raw beetroot showed in **Table (5).**The values were found to be (465.16± 0.86, 114.05± 0.15 and 19.36± 0.79 mg/100g,respectively).Ascorbic acid is a major element of human diet. It is a reality that ascorbic acid is engaged in the discharge of iron from ferritin **(Mazur *et al.*, 1955).** A reduced concentration of ascorbic acid and its reduced catabolism is also a condition found during anemia **(Jacobs *et al.*, 1971).** Beetroot is a good source of phytochemical compounds, including ascorbic acid, flavonoids, phenolic acids and carotenoids **(Wootton-Beard and Ryan, 2011). Kale *et al.* (2018)** found that the ascorbic acid and betalain content of beetroot juice were 10.01 and 14.20 mg/100g respectively.

Kujala *et al.* (2001) noted that beetroot includes significant amounts of phenolic acids such as phydroxybenxoic, syringic acids, vanillic, ferulic, p-coumaric, and protocatechuic**.** Also, **Ogan *et al.* (2004)** reported that beetroot includes important amounts of phenolic acids such as cinnamic acid, ferulic acid, p-coumaric acid, chlorogenic acid and caffeic acid in addition to small amounts of vitamin B12, vitamin C, vitamin A, zinc, sodium, potassium, iron and calcium. **Guldiken *et al.* (2016)** evaluated and compared total flavonoid content (TF), the total phenolic (TP), total antioxidant capacity (TAC) of fresh, pickled, boiled, pureed, juice, oven-dried, jam red beetroot and found that dried, fresh

and pureed red beetroot samples had the highest TP, TAC and TF. Moreover**, Ramos *et al.* (2017)** examined the effect of cooking method (Steaming, Pressure, Oven-baked) on the content of phenolic compounds and found that there was no important distinction in the phenolic compounds concentration between uncooked and cooked beets. This can be clarified by the enhanced phenolic compounds extraction from the cell matrix owing to modifications in texture during cooking **(Blessington *et al.*, 2010).**

Ramos *et al.* (2017) revealed greater concentrations of flavonoids in raw beet (290.64 mg rutin 100 g^{-1}). Also, **Koubaier *et al.* (2014)** found that the presence of three flavonoids (myricetin, kampferol, and quercetin) for roots of red beet by using Liquid chromatography–mass spectrometry. Moreover, **El-Beltagi *et al.*(2018**) found that the ethanolic extract of red beetroot contains a number of flavonoids compounds such as myricetin (19.3 mg/100g DW), neringenin (19.9 mg/100g DW), kaempferol (3.0 mg/100g DW) and apigenin (2.56 mg/100g DW).

In this research, the scavenging activity was determined by the DPPH test method, which was observed to be fast, simple and economical to measure antioxidant activity (**Rahman *et al.*, 2012**). The DPPH of raw beetroot was 72.64± 0.78 % and the value of IC_{50} was 2.75± 0.25 mg/ml. The total antioxidant activity and the value of IC_{50} showed in **Table (5)**.Compared to other vegetables, the antioxidant ability of the beet is very high. **Delgado *et al.* (2000)** observed that red pigment content in red beet could be as high as 500 mg/100 g on fresh weight. A extremely important connection was discovered between antioxidant and red pigment content, whereas a remarkably less concrete connection was discovered between antioxidant ability and yellow pigment content **(Czapski *et al.*, 2009).**

Ramos *et al.* (2017) found that antioxidant activity of raw beetroot was 57.63% measured by DPPH. **Jiménez-Monreal *et al.* (2009)** evaluated the effect of cooking techniques on antioxidant activity in vegetables and demonstrated that beet maintained its antioxidant activity when evaluated using many antioxidant activity quantitative techniques. Also, **Sawicki and Wiczkowski (2018**) found that red beet pulp and peels exhibits high antioxidant activity after heat treatment (10 min, 80˚C). While **Boari *et al.* (2013)** found that red beetroot boiling induced the greatest loss of the overall antioxidant activity.

Table (5): Bioactive components and antioxidant activity of beetroot

Parameters	Value
Total phenolic (mg/100g)	**465.16± 0.86**
Total flavonoids (mg/100g)	**114.05± 0.15**
DppH%	**72.64± 0.78**
IC_{50}	**2.75± 0.25**
V.C (mg/100g)	**19.36± 0.79**

Data was expressed using Mean ± SD. on dry weight basis.

Organoleptic evaluation of the beetroot products

Red beets are used in human consumption throughout the globe. The roots are used to make juice, salads, soups and jam **(Guldiken *et al.*, 2016)**.The primary sugar in beetroot, as opposed to fruit, is sucrose. The juice is taken as a natural therapy for sexual weakness and to remove stones of the kidney and bladder (**Sharma *et al.*, 2011).** In this study, beetroot was used in the preparation of soup, salad and pudding.

Data presented in **Table (6)** reveals that the beetroot juice prepared by boiling for 5 min possessed significantly($P\leq 0.05$) the highest score as judged by panelists. In contrast, raw beetroot juice (without any treatment) was ranked significantly($P \leq 0.05$) as the least acceptable one by panelists.

According to the present results, it can be concluded that boiling of beetroot pulp for a short period time (5 min) improved significantly ($P \leq 0.05$) the sensory quality attributes of beetroot juice. Therefore, this method has been used in the preparation of juice which used in the making of pudding and soup. **Wootton-Beard *et al.* (2011)** reported that different handling techniques applied to food products have important impacts on their antioxidant capacity and the bioaccessibility of phytochemicals included. Related Red Beetroot studies investigating the impacts of juice processing. Such an effect of boiling process is in accordance with published data that revealed various processing techniques of food, For example fermentation, grinding and/or light heating, may improve the bioactive phytonutrients bioaccessibility by breaking down the cell walls of plant tissues or nutrient matrix complexes or converting them into more effective molecular structures **(Parada and Aguilera, 2007).**

Table (6) shows that beetroot soup prepared by boiling beetroot for 5 min was significantly differed ($P\leq 0.05$) and the most acceptable treatment and superior to the control as judged by panelists. These results agree with our previous explanation of the effect of heating for short time to enhance

the availability of some components present in beetroot pulp and play a role in improving the sensory properties of beetroot.

It was obvious that boiling beetroot for 15 min before the preparation of salad was significantly ($P \leq 0.05$) superior to control group as it is shown in **Table (6)**. Accordingly, boiling of beetroot for up to 2 min is recommended to produce high quality (high acceptable) salad beetroot.

The preparation of pudding with different concentrations (i.e. 25, 50 and 75%) gave significantly ($P \leq 0.05$) different products from the sensory point of view (**Table 6**). The concentration of 25 % was found to be significantly ($P \leq 0.05$) the most acceptable treatment. It is worth to note that the control was significantly ($P \leq 0.05$) more acceptable than the other treatments applied in the present study (i.e. 25, 50 and 75%). The latter treatment was found to be significantly ($P \leq 0.05$) the least acceptable one because the surface colour was darker as the level of beetroot juice increased. In a similar study **Attia *et al.* (2013)** prepared jelly by incorporating natural color from red beet at 0.30 percent had the largest score of tested attributes followed by adding 0.4 and 0.2 respectively. On the other side, the ice sherbets prepared by adding betalain derived from red beet 0.20 % had a largest score of investigated attributes followed by the addition of 0.30, 0.10, 0.40 and 0.50% respectively.

Also, **Ingle *et al.* (2017)** found that the replacement of flour with beetroot powder by 10 % had a greater average color and appearance score. The ground color was darker as the amount of beetroot powder rose. Therefore, replacing up to 10 percent of wheat flour with beetroot powder led to excellent acceptability of cookies.

Table (6): Organoleptic evaluation of beetroot products

Products		Organoleptic evaluation			
		Color	Flavor	Texture	Overall acceptance
Beetroot Juice*	Control	3.72±0.11 ^{d}	3.55± 0.32 ^{d}	3.28± 0.04 ^{d}	3.60± 0.67 ^{c}
	BJ (3)	6.65± 0.73 ^{b}	6.88± 0.60 ^{b}	7.30± 0.33 ^{b}	6.55± 0.72 ^{b}
	BJ (5)	8.45± 0.52 ^{a}	8.35± 0.75 ^{a}	8.47± 0.18 ^{a}	8.07± 0.59 ^{a}
	BJ (8)	5.70± 0.08 ^{c}	5.63± 0.64 ^{c}	5.45± 0.23 ^{c}	5.90± 0.46 ^{b}
Beetroot soup**	Control	7.15±0.31 ^{b}	6.28± 0.71 ^{b}	6.95± 0.40 ^{b}	6.79± 0.53 ^{b}
	Treatment	8.72± 0.83 ^{a}	8.81± 0.85 ^{a}	9.10± 0.66 ^{a}	8.89± 0.02 ^{a}
Beetroot salad***	Control	7.18± 0.78 ^{b}	6.71± 0.33 ^{b}	6.15± 0.42 ^{b}	6.92± 0.89 ^{b}
	Treatment	8.55± 0.20 ^{a}	8.39± 0.65 ^{a}	8.47± 0.13 ^{a}	8.56± 0.30 ^{a}
Beetroot pudding	Control	8.50±0.53 ^{a}	9.11± 0.89 ^{a}	8.31± 0.28 ^{a}	8.99± 0.68 ^{a}
	25%	7.97±0.84 ^{ab}	8.08±0.61 ^{ab}	8.12± 0.13 ^{a}	8.35±0.64 ^{ab}
	50%	7.13±0.69 ^{bc}	7.16±0.24 ^{bc}	7.50± 0.89 ^{a}	7.53± 0.04 ^{b}
	75%	6.63± 0.06 ^{c}	6.46± 0.84 ^{c}	7.34± 0.59 ^{a}	6.26± 0.37 ^{c}

Means of each product in the same **column** with **small common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$).

*__Control Juice__: raw Juice, **BJ (3)**: beetroot juice boiling for 3 min, **BJ (5)**: beetroot juice boiling for 5 min, **BJ (8)**: beetroot juice boiling for 8 min.**__(control soup):__ boiling for 35 min, Treatment: boiling for 5 min.***__(Control salad) :__ boiling for 35 min, Treatment: boiling for 15 min.

Results of animal experiments

Effect of beetroot products on plasma blood picture of healthy and anemic rats.

The effect of beetroot products on Hematocrit (HCT) , hemoglobin (Hb) , red blood cell (RBC), meancorpuscular volume (MCV), Mean Cell Hemoglobin (MCH),mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBCs) of healthy and anemic rats shown in **Table** (7). Treatment with salad, pudding and soup in healthy male rats showed an increase in HB, RBC, HCT, MCV, MCH, MCHC and WBCs as compared to the negative control group. While, treatment with Phenylhydrazine alone caused a significant ($P \leq 0.05$) decrease in HB, RBC,HCT, MCV, MCHC and WBCs. On the other hand, the presence of salad, pudding and soup with (PHZ) led to an improvement in HB, RBC, HCT, MCV, MCH, MCHC and WBCs blood level.

Injection of PHZ induced a significant inhibition ($P \leq 0.05$) in RBC as compared to negative control group. This is in agreement with **McMillan *et al.* (2005)** who stated that phenylhydrazine caused the destruction of red blood cells (RBCs). During the process of oxidation, it forms free radicals**.**When it enters the bloodstream, it triggers hemolysis owing to oxidative changes in blood cell proteins **(McMillan *et al.*, 2005).** This process leads to premature aging of erythrocytes and predisposes to premature splenic sequestration. This results in a lack of hemoglobin and circulating erythrocytes **(Magnani *et al.*, 1988).** PHZ is absorbed by the inhalation, oral and dermal routs. After absorption it creates oxidative stress in RBCs and produces reactive oxygen species (ROS) in RBCs, ROS responds with haemoglobin and changes the oxyhaemoglobin in to hemichromes, methaemoglobin and other haemoglobin breakdown products such as Heinz bodies. This compound appears to be very helpful in models studying mechanism of hemolytic anemia **(Pandey *et al.*, 2014)**. It also caused an increases in the MCH, MCHC , MCV and extra medularhaemato poiesis in the liver and spleen **(Unami *et al.*, 1996**).

A supplementation of beetroot salad, pudding and soup to rats showed a significant enhancement at $P \leq 0.05$ in HB and RBC in plasma as compared to negative control group. Also, in rats given salad, pudding and soup during treatment with PHZ a significant elevation ($P \leq 0.05$) in HB and RBC in the plasma compared to the PHZ group was observed. This is due to the high level of iron in beetroot .Iron supplementation was correlated with a substantial rise in the mean ferritin, serum iron, and Hb together with the biomarkers of oxidative stress (MDA) and systemic inflammation indexes (NLR) and absolute counts of monocyte, neutrophil, and lymphocyte **(Aly *et al.*, 2016).** This is in agreement with **Al-aboud (2018)** who found that hemoglobin and ferritin had risen for 20 days after taking 8 g of beetroot, and therefore it can be argued that beetroot may have some therapeutic characteristics for iron deficiency.

In another research undertaken by **Jaiswal *et al.* (2014)** found that beetroot (200 mg / kg) proved an anti-anemic impact by considerably

increasing RBC and Hb levels in anemic rats treated with beetroot compared to anemic phenylhydrazine-induced rats. The present results are consistent also with the previous findings of **Beshel *et al.* (2018)** who found that beetroot at 100 and 400mg / kg was capable of preventing the effects of phenylhydrazine because of an rise (68% and 87% respectively) in RBCs in beet groups relative to the anemic group. Also**, Indhumathi and kannikaparameswari (2012)** recorded a substantial rise in dose dependent HB concentration in ordinary rats treated with methanolic beetroot extract (100, 200 and 400 mg / kg) administered for 16 days. This shows the tendency of beetroot to avoid anemia of iron deficiency. Beetroot includes folic acid and folic acid is essential for the absorption of iron from the gastrointestinal tract for HB synthesis. The high dose of beetroot not only avoided the adverse impact of phenylhydrazine on HB but also likely enhanced HB biosynthesis as the HB concentration was considerably increased in the beet group compared to the control groups **(Beshel *et al.*, 2018).**

Table (7):Effect of beetroot products on plasma blood picture of healthy and anemic rats

	Control (-) (n = 10)	Control(+) (n = 10)	Salad(-) (n = 10)	Pudding(-) (n = 10)	Soup(-) (n = 10)	Salad(+) (n = 10)	Pudding(+) (n = 10)	Soup(+) (n = 10)
HCT	43.2 ±0.3 ^{d}	12.4 ±0.21 ^{h}	45.3 ±0.14 ^{c}	45.7 ±0.21 ^{b}	51.2 ±0.16 ^{a}	28.9 ±0.05 ^{g}	38.5 ±0.1 ^{f}	39.3 ±0.1 ^{e}
HB(g/dl)	13.98 ±1.0 ^{d}	4.87±0.15 ^{h}	14.80 ±0.08 ^{c}	15.60 ±0.18 ^{b}	16.35 ±0.06 ^{a}	11.08 ±0.09 ^{g}	12.05 ±0.14 ^{f}	13.02 ±0.05 ^{e}
RBC(10^{6}/cmm)	4.42 ±0.4 ^{d}	1.49 ± 0.12 ^{h}	4.62 ±0.03 ^{c}	4.73 ±0.13 ^{b}	5.23 ±0.05 ^{a}	3.52 ±0.02 ^{g}	3.89 ±0.05 ^{f}	4.05 ±0.01 ^{e}
MCV	97.7 ±0.7 ^{d}	83.2 ±0.25 ^{g}	98.0 ±0.16 ^{b}	96.6 ± 0.22 ^{f}	97.8 ±0.17 ^{c}	82.1 ±0.1 ^{h}	98.9 ±0.2 ^{a}	97.03 ±0.01 ^{e}
MCH	31.6 ±0.6 ^{e}	32.68 ± 0.05 ^{a}	32.03 ±0.03 ^{d}	32.09 ±0.11 ^{c}	31.26 ±0.17 ^{g}	31.47 ±0.09 ^{f}	29.97 ±0.04 ^{h}	32.14 ±0.05 ^{b}
MCHC(g/dl)	32.2 ±0.2 ^{f}	39.27 ±0.06 ^{a}	32.67 ±0.21 ^{e}	34.13±0.1 ^{c}	31.9 ±0.03 ^{g}	38.3 ±0.08 ^{b}	31.46 ±0.03 ^{h}	33.63 ±0.11 ^{d}
WBCs(10^{3}/cmm)	4.8 ±0.8 ^{g}	3.9 ±0.1 ^{h}	5.2 ±0.06 ^{d}	5.6 ±0.2 ^{c}	4.9 ±0.51 ^{f}	5.0 ± 0.2 ^{e}	5.8 ±0.15 ^{b}	6.0 ±0.25 ^{a}

Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$). Hematocrit (HCT) , hemoglobin (HB) , red blood cell (RBC), meancorpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBCs). Data was expressed using Mean ± SD.

Effect of beetroot products on plasma glucose, total protein, albumin and globulin of healthy and anemic rats

The findings of glucose, total protein (TP), albumin and globulin of healthy and anemic rats are tabulated in **Table (8)**. Treatment with salad, pudding and soup in healthy male rats showed an improvement in plasma levels of TP, albumin and globulin compared to the negative control group. While, treatment with phenylhydrazine alone caused a significant ($P \leq 0.05$) decrease in plasma total protein (TP), albumin and globulin. On the other hand, the presence of salad, pudding and soup with (PHZ) increased the concentration of plasma TP, albumin and globulin. The treatments of rats with (PHZ) led to an increase in glucose level but the use of beetroot products led to an improvement in glucose blood level. This study showed that after using beetroot soup, there was a significant

($P \leq 0.05$) increase in total protein (TP), globulin and albumin compared to other products.

The present results showed that (PHZ) decreased plasma proteins and albumin. These findings are in consistent with those obtained by **McMillan *et al.* (1998)** who found that PHZ causes ATP depletion, lipid peroxidation, damage in skeletal protein, cation imbalances, and reduced membrane deformability. All these symptoms indicate hemolytic anemia. The commonly researched studied oxidative stress caused proteins modification is the production of carbonyl derivatives on histidine, arginine, proline, lysine, threonine, and cysteine residues**.** Total protein concentration is probable to decrease if protein synthesis is inhibited or protein degradation is encouraged (**Heidenreich *et al.*, 1999).**

Venkatesan *et al.* (2000) proposed that the concentration of plasma albumin could be immediately changed as a consequence of the loss of albumin due to damaged glomeruli in the case of kidney failure. As a result, the important reduction in albumin in this research may be linked to PHZ-induced nephrotoxicity, but the presence of beetroot salad, pudding and soup with PHZ alleviated its toxic effects on kidney. The significant alleviation by the beetroot products may be linked to the beetroot potential to alleviate this nephrotoxicity.

The present study showed that beetroot products decreased plasma glucose. This is consistent with the results of **Wooton-Beard *et al.*(2011**) who reported that early insulin response was found in healthy volunteers to lower their glucose concentrations after ingestion of beetroot juice. It also agrees with the work of **Gilchrist *et al.* (2014**) who found that after using beetroot juice, the response time for diabetic type 2 people has improved. This anti-glycaemic impact could be ascribed to one of the many antioxidant compounds contained in beetroot especially alpha-lipoic acid, which increases insulin sensitivity (**Gilchrist *et al.*, 2014**).

The impact of red beet on the metabolism of carbohydrates, in specific the mechanics of glycemia, both in ordinary circumstances and in diabetes, is being actively investigated. Based on the biochemical and morphological outcomes acquired in the streptozotocin diabetic rat experiment, an extract of beetroot, when administered by tube feeding, decreases blood glucose concentrations through pancreatic beta cells regeneration **(Bolkent *et al.*, 2000).** Usage of soluble dietary fiber is associated with reduced insulin reactions and postprandial glucose and therefore has positive impacts on metabolic syndrome.

It has been found that beetroot contains a significant amount of phenolic acids such asp-coumaric acid, chlorogenic acid, cinnamic acid, caffeic acid, ferulic acid and in addition to small amount of vitamin C, vitamin B12, vitamin A, potassium, calcium, sodium, zinc and iron **(Ogan *et al.*, 2004).** However, polyphenols are known to modulate the expression and transcription of proteins linked to endogenous antioxidant protection by interacting with antioxidant

response elements in gene promoter areas of genes encoding proteins linked to oxidative injury management **(Moskaug *et al.*, 2005).**

From the present results the administration of the beetroot soup, salad and pudding showed slight differences in total protein, globulin and albumin. This may be due to the same content of antioxidant in the beetroot products. **Melo *et al.* (2009)** analyzed the antioxidant activity of a variety of vegetables undergoing heat treatment and demonstrated that cooking did not significantly influence their antioxidant characteristics. This may be related to compensation by newly formed compounds, like the maillard response, or because a compound with partial oxidation to donate hydrogen atom to the hydroxyl radical and/ or the polyphenol aromatic structure tolerates the displacement of unpaired electrons around the ring **(Nicoli *et al.*, 1999).**

Table (8): Effect of beetroot products on plasma glucose, total protein, albumin and globulin of healthy and anemic rats

	Control (-) (n = 10)	Control (+) (n = 10)	Salad (-) (n = 10)	Pudding (-) (n = 10)	Soup (-) (n = 10)	Salad (+) (n =10)	Pudding (+) (n = 10)	Soup (+) (n = 10)
Glucose	124.5±0.37 ^{e}	168.8 ±0.28 ^{a}	123.6 ±0.43 ^{f}	121.6 ±0.41 ^{g}	120.0 ±0.65 ^{h}	140.2±0.11 ^{b}	135.3 ±0.71 ^{c}	128.7±0.58 ^{d}
TP	7.98±0.59 ^{b}	6.50 ±0.23 ^{d}	8.60±0.56 ^{a}	8.65±0.30 ^{a}	8.84 ±0.69 ^{a}	7.23 ±0.60 ^{c}	7.76±0.31 ^{bc}	7.93±0.52 ^{b}
AL	5.39±0.43 ^{b}	4.22±0.25 ^{d}	5.87±0.41 ^{a}	5.92±0.21 ^{a}	6.12±0.31 ^{a}	4.69 ±0.70 ^{c}	4.72±0.45 ^{c}	5.10 ±0.19 ^{bc}
GL	2.59±0.17 ^{de}	2.28 ± 0.02 ^{e}	2.73±0.09 ^{bc}	2.73 ±0.39 ^{bc}	2.72 ±0.16 ^{bc}	2.54 ±0.11 ^{cd}	3.04 ±0.14 ^{a}	2.83 ±0.33 ^{ab}

Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$) . **Data was expressed using Mean ± SD.** Total protein (TP), albumin (AL), globulin (GL)

Effect of beetroot products on plasma lipid profile of healthy and anemic rats.

Lipid profile which includes measurement of total lipids (TL), cholesterol, triglycerides (TG), high and low density lipoprotein-cholesterol (HDL-C and LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) are summarized in **Table (9)**. Treatment with PHZ alone resulted in a substantial rise ($P \leq 0.05$) in plasma TL, cholesterol, TG, LDL-C and VLDL-C, while HDL-C was decreased ($P \leq 0.05$) compared to the negative control group. On the other hand, treatment with salad, pudding and soup in healthy rats groups induced a substantial reduction ($P \leq 0.05$) in the concentration of all plasma lipid profile, except HDL-C which showed a significant increase ($P \leq 0.05$) in male rats compared to the negative control group. Additionally, the presence of salad, pudding and soup with PHZ in combination groups returned the values of the previous parameters close to the negative control group.

The increase in plasma lipid profile of male rats treated with PHZ alone Which leads to anemia in the present study is in agreement with **Tanzer *et al.* (2001)** who showed that lipid levels rise in iron deficiency in some studies of humans and animals. Also, **Ohira *et al.* (1980)** proposed that the level of red blood cells may influence the synthesis of cholesterol or the mobilization from tissue to plasma. Lipid parameters improve with the treatment of iron deficiency anemia, according to the outcomes of this research. These findings support the assumption that the extra protective function of iron deficiency states is connected with mechanisms involved in lipid metabolism **(Ozdemir, *et al.*, 2007).**

Decreased plasma lipid profile of male rats treated with beetroot salad, pudding and soup which showed in the present results is in agreement with **Bobek *et al.* (2000**) Who found that red beet fiber led to a 30 % decrease in cholesterolemia. This decline could be ascribed to the shift in LDL at 60 %, while the contribution of HDL to the transport of cholesterol was considerably improved. Red beet fiber did not change the cholesterol content of the liver and heart; however, its aorta content was considerably decreased by almost 30%. Red beet fiber diet also led to a decrease in serum triacylglycerols. The most significant stage in the mechanism of the hypocholesterolemic impact of the fiber is its capacity to bind bile acids resulting in decreased formation of micelain and sequentially decreased absorption of cholesterol. Enhanced excretion of bile acids restricts their enterohepatial circulation. This in turn stimulates the activity of 7ahydroxylase by a feedback mechanism and therefore increases the catabolism of cholesterol **(Vahouny, 1980).**

Also**, Sadeek (2011)** noticed that oral administration of fresh red beetroot juice considerably reduced TC serum, TG, VLDL and LDL-C. A substantial increase happened in the HDL-C level in rats treated with red beetroot. Phenolic compounds and betalains present in red beetroot have been reported to improve opposition of low density lipoproteins (LDL) to oxidation and to prevent cancer and cardiovascular diseases by decreasing the oxidative impact of free radicals on lipids **(Singh and Hathan, 2014).**

The presence of beetroot salad, pudding and soup with PHZ, especially soup in combination groups caused a decrease in plasma lipid profile of male rats, Probably because of their high antioxidant content. **Singh *et al.* (2015b)** observed that cooking techniques involving hot water immersion resulted in an rise in carotenoid content in pumpkin, taro and beet. These modifications could be due to enhanced carotenoid extraction ability in the cellular system by boiling in water. **Miglio *et al.* (2008)** noted an rise in the antioxidant capacity of broccoli, zucchini and carrots after cooking in water, frying and steaming. According to this research, this outcome was most probable owing to the softening of the vegetable matrix and enhanced extractability of the

compounds, which could be partly transformed into more antioxidant chemical species. The authors also indicate that the carotenoid content of the three vegetables and ascorbic acid in zucchini and carrots is better maintained by immersion in water treatment. This lipid lowering potential of beetroot salad, pudding and soup may be due to flavanoids and/or saponins which were found to be the main constituents of beetroot in our preliminary phytochemical screening. These findings are consistent with earlier studies showing the effect of flavonoids on the metabolism of cholesterol (**Hostettman and Marston, 1995).** therefore, natural products with a beneficial impact on human health could demonstrate to be more effective therapy due to its capacity to considerably increase HDL-C while reducing total cholesterol.

Brown *et al.* (2018) reported considerably reduced concentrations of lipid (TC, TG and HDL) in the test group after ingestion of 300 g of carbohydrate meal and 250 ml of beetroot juice relative to pre-treatment lipid levels. There were no important variations in pre-and post-treatment lipid levels in the control group, suggesting that beetroot juice reduced lipid levels in the test group. This agrees with the work of **Singh *et al.* (2015a)** in which supplementation of beetroot juice reduced LDL cholesterol concentrations in physically active people.

Table (9): Effect of beetroot products on plasma lipid profile of healthy and anemic rats

	Control (-) (n = 10)	Control(+) (n = 10)	Salad(-) (n = 10)	Pudding(-) (n = 10)	Soup(-) (n = 10)	Salad(+) (n = 10)	Pudding(+) (n = 10)	Soup(+) (n = 10)
TL	520.25± 0.76 ^{e}	586.5 ± 0.62 ^{a}	450.2± 0.70 ^{f}	442.7 ± 0.79 ^{g}	430.6 ± 0.33 ^{h}	560.8 ±0.07 ^{b}	552.7± 0.61 ^{c}	539.4± 0.04 ^{d}
Cholesterol	156.5 ± 0.59 ^{e}	201.0± 0.29 ^{a}	136.0 ± 0.33 ^{f}	131.02 ±0.41 ^{g}	126.2 ± 0.40 ^{h}	185.1 ± 0.44 ^{b}	171.0± 0.54 ^{c}	168.4± 0.60 ^{d}
TG	123.7± 0.24 ^{e}	155.4 ± 0.71 ^{a}	97.70 ± 0.05 ^{f}	95.30 ± 0.01 ^{g}	89.22 ± 0.21 ^{h}	142.9 ± 0.80 ^{b}	138.5± 0.14 ^{c}	132.9 ± 0.03 ^{d}
HDL-C	55.57± 0.70 ^{d}	36.27 ± 0.42 ^{h}	67.61 ± 0.51 ^{c}	70.20± 0.23 ^{b}	76.61 ± 0.55 ^{a}	43.8 ± 0.62 ^{g}	46.78± 0.44 ^{f}	50.34± 0.45 ^{e}
LDL-C	76.19 ± 0.15 ^{e}	133.65 ± 0.28 ^{a}	48.85 ± 0.61 ^{f}	41.76 ±0.54 ^{g}	31.74 ± 0.23 ^{h}	112.72 ± 0.32 ^{b}	96.52± 0.50 ^{c}	91.48 ± 0.15 ^{d}
VLDL-C	24.73 ± 0.05 ^{e}	31.08 ± 0.14 ^{a}	19.54 ± 0.01 ^{f}	19.06 ±0.0 ^{g}	17.84 ± 0.04 ^{h}	28.59 ± 0.16 ^{b}	27.70± 0.03 ^{c}	26.59 ± 0.01 ^{d}
AI	1.82 ± 0.18 ^{e}	4.54 ± 0.56 ^{a}	1.01 ± 0.01 ^{f}	0.86 ± 0.05 ^{fg}	0.65 ± 0.08 ^{g}	3.22± 0.10 ^{b}	2.65± 0.29 ^{c}	2.34 ± 0.02 ^{d}

Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$). **Data was expressed using Mean ± SD.**
Total lipids (TL), Triglycerides (TG), High density lipoprotein -cholesterol (HDL), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C).

Effect of beetroot products on plasma (TBARS) and the antioxidant enzymes activity of healthy and anemic rats.

The results of plasma (TBARS) and the antioxidant enzymes activity of healthy and anemic rats are tabulated in **Table (10)**. The results indicated that the use of PHZ alone significantly ($P \leq 0.05$) increased TBARS in plasma compared to the negative control group. While, it caused a significant ($P \leq 0.05$) reduction in the levels of GSH and the

activities of GPx, SOD, CAT and GST in plasma compared to the negative control group. On the other hand, treatment with salad, pudding and soup in healthy rats groups caused a significant ($P \leq 0.05$) decrease in TBARS concentration and increase in GSH, GPx, GST, CAT and SOD in plasma of rats. Also, the presence of salad, pudding and soup with PHZ in combination groups minimized its toxic effect compared to PHZ group.

Thiobarbituric acid reactive substances (TBARS) are generated by peroxidation of lipid and are regarded oxidative stress indicators **(Karthikeyan *et al.*, 2007).**When ROS begin to accumulate, cells have a defensive mechanism that uses a variety of antioxidant enzymes. The main detoxifying systems for peroxides are GPx, CAT, GSH, GST, and SOD.

Injection of PHZ induced a significant inhibition ($P \leq 0.05$) in SOD, GST, GPx, GSH,CAT activities and increase in the level of (TBARS) in plasma relative to the negative control group. This is in agreement with **Pandey *et al.* (2014)** who indicated that PHZ induced the development of reactive oxygen species resulting in lipid peroxidation and oxidative degradation of spectrin in the Skelton membrane. A supplementation of salad, pudding and soup to rats showed a significant enhancement ($P \leq 0.05$) in SOD, GST, GPx, GSH and CAT activity in plasma as compared to negative control group. In rats given salad, pudding and soup during treatment with PHZ a significant elevation ($P \leq 0.05$) in both enzymes activities in the plasma compared to the PHZ group was observed. This results indicated that, the potent antioxidant activity of beetroot may be related to it's phenolic compounds and flavonoids. some flavonoids are known to decrease xenobiotic-induced hepatotoxicity in animals and to counteract the harmful impacts of oxidative stress by cooperating with natural structures such as glutathione and other endogenous protective enzymes (**Kadarian *et al.*, 2002).** This was expected and confirmed by the findings of **Váli *et al.* (2007)** who reported that beet contains major bioactive agents (polyphenols and betaine) with a broad spectrum of physiological impacts. Beetroot is one of the few vegetables that contains a group of extremely bioactive pigments known as betalains. **(Vulić *et al.*, 2014).** Betalains are water-soluble plant pigments that are commonly used as food colorants and have a broad variety of desirable biological activities, including hepato-protective, anti-inflammatory, antioxidant, anti-cancer characteristics **(Georgiev *et al.*, 2010).**

From the present results the administration of the soup showed an insignificant increase in plasma GSH, SOD, CAT, GST, and GPx compared to the salad and pudding. This may be due to the high content of antioxidant in the component of soup. **Bobek *et al.* (2000)** proved that red beet fiber had a substantial rise in the activity of CAT and SOD in the liver of colon, CAT, GSH-PX and GST. The decrease of TBARS in plasma after using salad, pudding and soup (**Tables 10**) is in agreement with results of **Kiran *et al.* (2013)** who found that food rich in flavonoids, anthocyanins and polyphenols

have been shown to be efficient in scavenging free radicals. Also, **Kujawska *et al.* (2009)** found that pretreatment with juice resulted in a partial recovery of glutathione reductase and glutathione peroxidase activity by 66% and35%, respectively.

Table (10): Effect of beetroot products on plasma (TBARS) and antioxidant enzymes activity of healthy and anemic rats

	Control (-) (n = 10)	Control(+) (n = 10)	Salad(-) (n = 10)	Pudding(-) (n = 10)	Soup(-) (n = 10)	Salad(+) (n = 10)	Pudding(+) (n = 10)	Soup(+) (n = 10)
GSH	52.50± 0.48 ^{d}	23.50 ± 0.16 ^{h}	58.90± 0.19 ^{c}	67.30± 0.08 ^{b}	71.40 ± 0.36 ^{a}	44.70± 0.55 ^{g}	46.50± 0.0 ^{f}	49.80±0.33 ^{e}
GST	0.70± 0.21 ^{bc}	0.35± 0.07 ^{c}	1.09± 0.18 ^{a}	1.10± 0.40 ^{a}	1.10± 0.49 ^{a}	0.75± 0.23 ^{ab}	0.82± 0.09 ^{ab}	0.85± 0.57 ^{ab}
TBARs	1.91± 0.39 ^{b}	2.60± 0.63 ^{a}	1.38± 0.29 ^{c}	1.35± 0.14 ^{c}	1.30 ± 0.28 ^{a}	2.08± 0.68 ^{b}	2.06± 0.37 ^{b}	2.03± 0.13 ^{b}
CAT	52.63± 0.53 ^{d}	22.70± 0.32 ^{h}	63.50± 0.67 ^{c}	69.20± 0.06 ^{b}	74.82± 0.31 ^{a}	41.65± 0.77 ^{g}	44.50± 0.18 ^{f}	48.63±0.22 ^{e}
SOD	1.60± 0.09 ^{b}	0.50± 0.13 ^{d}	2.04± 0.36 ^{a}	2.08± 0.64 ^{a}	2.12 ± 0.15 ^{a}	1.06 ± 0.38 ^{c}	1.11± 0.58 ^{c}	1.28± 0.25 ^{bc}
GPx	16.52 ± 0.54 ^{d}	7.36± 0.28 ^{h}	23.38± 0.38 ^{c}	24.20± 0.67 ^{b}	27.60± 0.38 ^{a}	11.99± 0.03 ^{g}	12.92± 0.23 ^{f}	15.25±0.13 ^{e}

Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$). **Data was expressed using Mean ± SD.** Glutathione (GSH), glutathione peroxidase (GPx),superoxide dismutase (SOD), catalase (CAT)and glutathione S transferase (GST).

Effect of beetroot products on plasma liver enzymes of healthy and anemic rats

The results of plasma liver enzymes of healthy and anemic rats are tabulated in **Table (11)**. Treatment with PHZ alone showed a significant ($P \leq 0.05$) increase in plasma AST, ALP, ALT and ACP compared with the negative control group. On the other hand, treatment with salad, pudding and soup in healthy rats groups showed a decrease in the plasma concentration of ALT, AST, ALP and ACP. The presence of salad, pudding and soup with PHZ in the combination groups caused a decrease in the elevated serum levels of plasma AST, ALT, ALP and ACP compared to PHZ group but did not reach the values of the negative control group.

The significant increase in plasma biomarker enzymes (AST, ALT, ALP and ACP) of rats treated with PHZ indicate the hepatocellular dysfunction and the severity of the liver necrotic damage. The results of the present study are in accordance with the study of **Pandey *et al.* (2014)** who studied the hepatic expression of several appropriate genes following PHZ induced haemolysis and observed that expression of TFR1 in liver was considerably increased in PHZ treated mice, while the expression of hepcidin decreased.

The decrease of plasma AST, ALT, ALP and ACP in the presence of beetroot products is in agreement with **Sadeek (2011)** who found that oral

administration of fresh juice from red beetroot and radish reduced considerably ($p \leq 0.05$) the serum ALP, ALT, AST and total bilirubin. Also, **Coles and Clifton, (2012)** indicated that beetroot juice may help reduce blood pressure and prevent damage of liver if it is included in the diet. **Krajka-Kuzniak *et al*. (2012)** examined the impact of long term feeding (28 days) of beetroot juice on phase I and phase II enzymes, DNA damage and liver damage caused by hepato-carcinogenic N-nitrosodiethyamine in rats. Long term feeding with beetroot juice gave the protective against oxidative damage of liver.

In PHZ groups treating animals with beetroot products especially soup gave the best results for all the enzymes compared to PHZ group. The dose of beetroot salad, pudding and soup effectively restored the functional integrity of liver by stopping penetration of the toxin into the interior of the cell. The treatment avoided the development of oxygen free radicals and therefore the formation of peroxy radicals. The efficacy of any hepatoprotective drug depends on its ability to either reduce the damaging impact or restore normal hepatic physiology that has been disrupted by a hepatotoxin. Beetroot products decreased PHZ -induced elevation of the enzymes levels in tested groups, indicating the protection of structural integrity of hepatocellular membrane or the regeneration of damaged liver cells.

Table (11): Effect of beetroot products on plasma liver enzymes of healthy and anemic rats

	Control (-) (n = 10)	Control(+) (n = 10)	Salad(-) (n = 10)	Pudding(-) (n = 10)	Soup(-) (n = 10)	Salad(+) (n = 10)	Pudding(+) (n = 10)	Soup(+) (n =10)
AST	55.20± 0.54 ^{d}	82.40± 0.21 ^{a}	43.20± 0.72 ^{f}	41.14± 0.17 ^{g}	40.66± 0.03 ^{h}	62.30± 0.37 ^{b}	59.40± 0.04 ^{c}	52.39±0.25 ^{e}
ALT	47.20± 0.73 ^{e}	75.67± 0.17 ^{a}	41.89± 0.75 ^{f}	40.91± 0.72 ^{g}	37.38± 0.60 ^{h}	58.30± 0.04 ^{b}	54.28± 0.27 ^{c}	50.19±0.44 ^{d}
ALP	143.3± 0.15 ^{e}	218.0± 0.15 ^{a}	111.2± 0.36 ^{f}	102.2± 0.13 ^{g}	98.50± 0.04 ^{h}	170.7± 0.64 ^{b}	165.2± 0.14 ^{c}	159.7±0.13 ^{d}
ACP	12.0± 0.22 ^{d}	18.20± 0.35 ^{a}	10.08± 0.17 ^{e}	9.40± 0.21 ^{f}	8.18± 0.75 ^{g}	15.85± 0.25 ^{b}	13.04± 0.55 ^{c}	12.98±0.46 ^{c}

Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$). **Data was expressed using Mean ± SD.** Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP).

Effect of beetroot products on plasma kidney functions of healthy and anemic rats

The data in **Table (12)** shows the effect of beetroot products on urea and creatinine levels of healthy and anemic rats. Results indicated that the use of PHZ alone significantly ($P \leq 0.05$) increased urea and creatinine in plasma compared to the negative control group. On the other hand, treatments with beetroot salad, pudding and soup in healthy rats groups caused a significant ($P \leq 0.05$) decrease in urea and creatinine concentration in plasma of rats. Also, the presence of salad, pudding and

soup with PHZ in the combination groups minimized its toxic effect compared to PHZ group. Plasma urea and creatinine results, showed that soup consumption slightly decreased urea and creatinine more than salad and pudding. Also, in PHZ groups, treating animals with soup gave the best results concerning plasma urea and creatinine. This may be due to their high content of polyphenols, flavonoides and antioxidant activity. Food processing is essential from a nutritional viewpoint, because it can impact the content, bioavailability, and activity of both antioxidants and nutrients. Processing can lead to useful transformations, degradation of compounds, or loss of nutrients **(Melo *et al.*, 2009)**. Cooking vegetable tissues alters the physicochemical properties of the cell wall, which in turn affects their efficiency as dietary fiber **(Santos *et al.*, 2003).**

El Gamal *et al.* (2014) found that treatment of rats with the beetroot ethanolic extract (250 and 500mg/kg) considerably avoided elevation of serum uric acid, urea, total protein, and creatinine concentrations in a dose dependent manner.

Table (12): Effect of beetroot products on plasma kidney functions of healthy and anemic rats

	Control (-) (n = 10)	Control(+) (n = 10)	Salad(-) (n = 10)	Pudding(-) (n = 10)	Soup(-) (n = 10)	Salad(+) (n = 10)	Pudding(+) (n = 10)	Soup(+) (n = 10)
Urea	32.20± 0.46 ^{e}	45.60± 0.06 ^{a}	30.70± 0.64 ^{f}	28.70± 0.63 ^{g}	24.70± 0.46 ^{h}	41.31± 0.64 ^{b}	40.21± 0.74 ^{c}	38.21±0.55 ^{d}
Creatinine	1.12± 0.12 ^{ab}	1.39± 0.06 ^{a}	0.67± 0.28 ^{c}	0.66± 0.40 ^{c}	0.62± 0.40 ^{c}	0.85± 0.39 ^{bc}	0.82± 0.60 ^{bc}	0.80± 0.48 ^{bc}

Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$). **Data was expressed using Mean ± SD.**

CONCLUSION

Beetroot at the administered doses exhibited anti-anemic effect which was more potent in anemia. It is therefore effective in the treatment of anemia. Generally, beetroot has numerous medicinal properties such as anti-microbial, anti-cancer, anti-inflammatory, anti-hyperglycemic, anti-oxidant, anti-hypertensive, hepatoprotective and diuretic. Therefore, It should be incorporated as part of daily diet and used as nutritional therapy.

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استخدام بعض منتجات البنجر لتحسين بعض القياسات في الفئران المصابة بفقر الدم الناتج عن الفينيل هيدرازين

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فقر الدم هو مرض واسع الانتشار بين مختلف البلدان. يحدث فقر الدم نتيجة لانخفاض نمو كريات الدم الحمراء ، مما يؤدي إلى ظهور كمية كبيرة من كريات الدم الحمراء الغير مكتملة. تعد جذور البنجر أحد أهم الخضروات التي يتم تناولها في جميع أنحاء العالم وتستخدم كعامل تلوين طبيعي تقليدي في العديد من المطابخ. طبيا ، يستخدم البنجر كدواء تقليدي للوقاية من الأمراض المختلفة بما في ذلك فقر الدم والكبد وتصلب الشرايين وأمراض الكلى والقلب التاجي. تهدف هذه الدراسة الى تقييم إمكانية مكافحة فقر الدم الذى يسببه فينيل هيدرازين في الفئران باستخدام حساء البنجر والسلطة والبودنج. تم تقدير التركيب الكيميائي والنشاط المضاد للأكسدة لجذور البنجر ثم تم تحضير العديد من المنتجات من جذور البنجر، وتشمل السلطة والبودنج والحساء، واستخدمت لتغذية الفئران الصحية والمصابة بفقر الدم لمدة 30 يومًا لمعرفة تأثير هذه المنتجات على بعض العوامل الحيوية النشطة .أظهرت النتائج أن جذور البنجر تحتوي على الرطوبة، البروتين، الدهون، الرماد ، الألياف الخام ، المستخلص الخالي من النتروجين والسعرات الحرارية بنسبة 84.57 ± 0.84 ، 13.02 ± 0.56 ، 0.16 ± 0.04 ، 15.49 ± 0.21 ، 3.65 ± 0.81 ، 67.68 ± 0.02 و 324.24 ± 0.55 ٪) ، على التوالي. وقد وجد أيضًا أن جذور البنجر غنية بالمركبات الفينولية ، والفلافونويدات، والنشاط المضاد للأكسدة. كشفت القيم أن منتجات جذور البنجر تسببت في تحسن في مستوى خلايا الدم الحمراء، الهيموجلوبين، MCV، MCH وMCHC في كل من الفئران السليمة والمصابة بفقر الدم. بالإضافة إلى ذلك، تسبب إطعام الفئران السليمة والمصابة بفقر الدم بمنتجات جذور البنجر في زيادة ملحوظة في البروتين الكلي، الألبومين والجلوبيولين مقارنة بمجموعة الفينيل هيدرازين. ومن المثير للاهتمام، أن التغذية بحساء البنجر والسلطة والبودنج أعادت نشاط الإنزيمات في بلازما الفئران السليمة والمصابة بالأنيميا بالقرب من مستوياتها الطبيعية. علاوة على ذلك، ادى استخدام منتجات جذور البنجر الى التقليل من التأثير الضار للفينيل هيدرازين على مستويات الدهون في البلازما وTBARS. توصى هذة الدراسة باستهلاك منتجات جذور البنجر للمرضى الذين يعانون من فقر الدم لتحسين مستويات الهيموجلوبين، ونسبة الدهون في الدم ، وأنزيمات مضادات الأكسدة ووظائف الكبد والكلى.

الكلمات المفتاحية: فقر الدم ، الفئران ، جذور البنجر ، فينيل هيدرازين ، مضادات الأكسدة.