

Effect of Feeding Iron Deficiency Anemia Rats on Red Beetroots Juices

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ABSTRACT

This investigation was aimed to study the effect of iron deficiency anemia rats feeding on red beetroot juice. The present investigation demonstrated that red beetroot could be comprises of mixes with the capacity to explicitly animate duodenal iron a ingestion. Red beetroot was analyzed for chemical compositions (ash, crude fats, crude proteins, crude fiber, carbohydrates, phenolic and flavonoids compounds). At feeding experiment was conducted on iron deficiency anemia rats fed on red beetroot. The outcomes demonstrated that iron-inadequacy caused many turn around impacts mirrored the noteworthy decrease of HCT, Hb, RBC, WBC, MCV and MCHC. On contrary, administration of ferrous sulphate (FeSO₄) and red beetroot juice induced a significant increase in Hb, HCT, RBC, WBC, MCV and MCHC values of serum iron profile.

Keywords: red beetroot- phenol compounds-flavonoids- Iron deficiency anemia.

INTRODUCTION

Iron deficiency anemia is referenced as the bringing down of the complete substance of iron in the body. Fe-insufficiency weakness happen when iron lack is amazingly extreme to diminish erythropoiesis. This sort of iron deficiency is the most incessant constant sickliness. Iron inadequacy might be the consequence of either intemperate misfortune, less oftentimes or diminished retention (Bermejo *et al.*, 2009). Anemia is a large public health problem in the worldwide with prevalence of 43% in developing countries and of 9% in developed nations (Habibzadeh, 2012). It is predominant in people at any phase of life, albeit little youngsters and pregnant-regenerative ladies are most powerless, which may expand the danger of physical improvement and augmentation grimmess and hindered intellectual and death rate (Khaskheli *et al.*, 2016). Reasons for the high spread wide of iron deficiency in industrialized and creating nations, it is important to keep up a reasonable Fe consumption through sustenance so as to accomplish a proper status of this component in the body. Hence, precise information of iron accessibility in weight control plans is major so as to design intercession systems that advance inadequate circumstances of this supplement (Lopez and Martos, 2004).

Red beetroot (*Beta vulgaris L*) is the taproot bit of the beet plant, otherwise called the nursery beet, brilliant table beet red beet or casually basically as the beet. The present addition consideration has been centered around usage of healthy foods. Red beetroot is generally cooked, but the beet root juice includes host of health benefits and is classed as a "super food" in today's. Some of the benefits of beetroots are relaxing smooth muscles, and lowering blood pressure by dilating the blood vessel rising the oxygen level, improving the stamina by decreasing the oxygen during exercise, treating anemia by increasing the blood count and improving blood circulation and oxygen carrying capacity of erythrocytes (red blood cells), preventing birth defects by folate and folic acid, Vitamin B12 together with folic acid, is necessary to form red blood cells. Beetroot juice is particularly beneficial as an anemia remedy for children and teenagers, beetroot juice or beet as cooked vegetable in salad is highly beneficial in treating anemia (Gayathri Priya *et al.*, 2013). Many explores demonstrated that beetroot could be added to improve the hemoglobin level in the blood. The expense is low when contrasted and other iron rich vegetables and it very well

may be put away effectively. Red beetroot incorporates an abnormal state of naturally open cell reinforcements. (Wootton-Beard *et al.*, 2011). as well as many other health promoting compounds such as folic acid, iron, magnesium, calcium, potassium, phosphorus and zinc. Beetroot is also high source of polyphenol components (Pitalua *et al.*, 2010). The beetroot being a soluble nourishment with pH from 7.50 to 8.00 has been soured for its medical advantages, in explicit for its ailment battling cancer prevention agent probability, noteworthy measure of vitamins B1, B2, niacin, B6, B12 and vitamins C (Singh and Singh Hatan, 2013).

Accordingly, the red beetroots was evaluated. The chemical composition, minerals content, phenolic and flavonoids compounds were determined. Feeding experiment was also conducted to study the effect of red beetroot against iron deficiency anemia.

MATERIALS AND METHODS

Materials

Red beetroots: were obtained from the local market at Kafrelsheikh City, Egypt.

Chemicals: Tannic acid, vitamins, casein, cellulose, minerals, choline chloride and methionine were gotten from El-Gomhoria Company. for Chemicals and Drugs, Cairo, Egypt.

Animals: 36 male albino rats (180-200g) weigh were brought from the Fac. of Veterinary Medicine Kafrelsheikh Univ., Egypt.

Methods:

Preparation of red beetroots Juice.

Red beetroots were washed under running water, peeled out and cut into pieces. Juice was then extracted using a "Moulinex electric juice extractor model 753" juice maker.

Chemical composition:

Moisture, crude protein, ash, ether extract, and crude fiber were determined according to the methods of the AOAC (2005). Total carbohydrates were calculated by difference as follows:-

$$\text{Total carbohydrates \%} = 100 - (\% \text{moisture} + \% \text{crude protein} + \% \text{ether extract} + \% \text{ash}).$$

Available carbohydrates were calculated by subtracting crude fiber from total carbohydrates, Minerals content was also determined the methods in the AOAC (2005).

Determination of phenolic and flavonoids compounds:

Phenolic compounds were fractionated and identified by High Performance Liquid Chromatography (HPLC), Hewlett Packard, series 1050 according to the method of Goupy *et al.*, (1999). Flavonoids compounds were determined according to Mattila *et al.*, (2000).

Biological assay:

Animals and experimental Design:

Male albino rats (n=36), weighing 180-200g, were put in plastic cages. The rats fed on the basal diet consisted of 20% protein (casein), 4.7% corn oil, 5% cellulose, 0.3% methionine, 0.2% choline chloride, 1% vitamin mixture, and 3.5% salt mixture and corn starch up to 100g (Reeves *et al.*, 1993). For seven days for acclimation a metallic tube tightly fixed at its mouth by a piece of rubber tube. Treatments and animal maintenance were in accordance with the guidelines of the experimental animal house of Food Technology Research Institute (FTRI), ARC Animal care and use committee, which follow the International Animal care and use Guidelines. All rats benefited from basal sustenance utilized in the analysis was set up after this period, rats were isolated into two primary groups. The principle gathering (six rodents) was benefited from basal eating routine as a negative control groups. The subsequent primary groups (30 rodents) was benefited from basal eating routine including tannic acid (20 g/kg body weight of rodents) for 3 weeks as suggested by Kaosar *et al.*, (2004). After that, six rats (G2) were continued benefiting from basal eating regimen as a positive control groups, while the others rats were separated into 4 groups. The third group (G3) fed on basal diets containing (ferrous sulphate 35 mg/kg diet) and the other groups from (G4 to G6) fed on basal diet with red beetroot juice (5, 10, 15 ml/kg body weight/day), respectively. It was orally using oral gavage for four weeks according to Rabeh (2015).

Group1: Rats fed on basal diet (negative control)

Group2: Rats fed on anemic diet (positive control)

Group3: Rats fed on anemic diet + ferrous sulfate (35 mg Fe/kg diet)

Group4: Rats fed on anemic diet + red beetroot juice (5 ml/kg body weight/day)

Group5: Rats fed on anemic diet + red beetroot juice (10 ml/kg body weight/day)

Group6: Rats fed on anemic diet + red beetroot juice (15 ml/kg body weight/day)

Body weight gain :-

The body weight gain was calculated according to Abdel Azim (2007) as follows:

$$\text{Body weight gain (BWG) \%} = \frac{(\text{Final weight} - \text{initial weight})}{\text{Initial weight}} \times 100$$

At finally of the exploratory, rats were fasted overnight at that point presented to ether anesthesia and blood tests were pulled back from eye plexus of veins into heparinized fine cylinders to test blood picture. In the wake of relinquishing rodents other blood tests were gathered from the hepatic gateway vein in dry rotator tubes. Serum was isolated by centrifugation of blood at 3000rpm (round every moment) for 10 minutes at room temperature at that

point moved into launder ebendorff cylinders and kept solidified at - 20°C till broke down.

Biochemical Analyses

Complete blood count (CBC) was determined using a graded scale Mean Corpuscular Volume (MCV). Mean Corpuscular Hemoglobin concentration (MCHC), red blood cells count (RBC) and white blood cell (WBC) were measured according to Fischbach (1996). Hemoglobin (Hb) and hematocrit (HCT) concentration was determined as mentioned by Dacie and Lewis, (1984).

Statistical Analysis

The outcomes data were displayed as means ± SD. The acquired information were factually investigated by the SPSS-PC (measurable bundle programming, form, 11.0). One route investigation of fluctuation (ANOVA) was utilized to test the contrasts among gatherings (Abou-Allam 2003).

RESULTS AND DISCUSSION

Chemical composition of red beetroot .

The compound piece of red beetroot is recorded in Table (1). The results indicated that red beetroot can be considered as a good source of carbohydrates, ether extract, crude protein, crude fiber and ash. It contains 86.7% moisture, 1.45% crude protein, 0.38% ether extract, 1.53% ash, 1.70% crude fiber and 8.24% available carbohydrates. These results are in near to those studied by Kale *et al.*, (2018). They reported that the red beetroots chemical composition values of moisture, protein, ether extracts, ash, crude fiber, and available carbohydrate were 87.40%, 1.35%, 0.30%, 1.40%, 1.90%, and 7.59% respectively.

Table 1. Chemical composition of red beetroots (as wet basis).

Constituents (%)	Red beetroots
Moisture	86.7
Crude proteins	1.45
Ether extracts	0.38
Ash	1.53
Crude fiber	1.70
Available carbohydrate	8.24

* Available carbohydrates were calculated by difference

Minerals content of red beetroots .

Results in Table (2) showed that red beetroots is rich in certain minerals such as Fe, K, Ca, Mn, Cu, Na, and Zn. These results are in similar with those of Kale *et al.*, (2018) who reported that beetroots are a rich source in calcium, potassium, sodium, iron, zinc, copper and manganese 12.20, 30.12, 72.58, 0.75, 0.21, 0.09 and 0.79 mg/100g respectively.

Table 2. Mineral Compositions of the red beetroot (as wet basis)..

Minerals	Red beetroot (mg/100g)
Iron (Fe)	00.78
Potassium (K)	31.16
Calcium (Ca)	13.20
Manganese (Mn)	00.74
Copper (Cu)	00.08
Sodium (Na)	70.79
Zinc (Zn)	00.20

Phenolic compounds and flavonoid compounds of red beetroot .

Phenolic and flavonoids compounds of red beetroot were separated and identified by High Performance Liquid Chromatography (HPLC) and the results are presented in Table (3). Seventeen phenolic compounds were identified. Pyrogallol was considered as predominant compound in red beetroot (328.63ppm), Ellagic acid (33.94ppm), Protocatchuic (32.78), Catechein(31.84), P-OH benzoic acid (26.81), benzoic acid (18.29), and Gallic acid (18.23) were common the phenolic compounds .The results are in similar with El-Dakak *et al.*, (2016), who showed that twelve mainly peaks of phenolic compounds which quantitatively determined. The peaks were assigned to pyrogallo, chlorogenic. epi-catechein, oleuropein, caffeic, ferulic vanillic, protocatchuic dihydroxy benzoic acid, catechin, salicylic acid and gallic acid. Flavonoids compounds are an important class of plant pigments naturally foods in plants. Therefore , the flavonoid compounds of red beetroot were separated and the results are presented in Table (3).Data reveal that, nine flavonoid compounds identified and hesperidin was the predominant flavonoid compound in red beetroot (252.89 ppm).

Biological assay

Body weight gain of rats fed on red beetroot juices.

Results in Table (4) indicated that, initial body weight of all group after adaptation on basal diet were nearly and with no significant differences .At the end of experiment periods the final body weights of anemic rats (G2) were lower than those of negative control (G1).

Anemic rats fed on basal diet and take the ferrous sulfate and juice (G3, G4, G5 and G6) had increased final body weight than those of the anemic control (G2). The obtained results illustrated that the body weight gain at the end of experiment periods for the negative control was (18.18), while the group positive control was (-7.69). while the anemic group Feeding of basal diet and take juices increase gain in body weight compared of G2. This decrease in body weight observed in iron deficiency anemia rats may be due to lower plasma thyroid hormone levels (Beard *et al.*, 1998).

Table 3. Phenolic and flavonoids compounds (ppm) content of red beetroot (on dry weight basis).

Phenolic compounds	ppm	Flavonoid compounds	Ppm
Pyrogallol	328.63	Rosmarinic	2.64
Gallic acid	18.23	Hesperidin	252.89
Protocatchuic	32.78	Rutin	2.97
Catechol	6.91	Quercetrin	8.33
4-Aminobenzoic acid	1.47	Naringenin	3.77
Catechein	31.84	Quercetin	1.63
Chlorogenic acid	9.88	Hespirtin	0.54
P-OH- benzoic acid	26.81	Kampferol	0.29
benzoic acid	18.29	Apigenin	0.44
Caffeic acid	3.28		
P-Coumaric acid	1.17		
Ferulic acid	3.66		
Iso-Ferulic acid	5.95		
α Coumaric acid	2.02		
Coumarin	2.37		
Ellagic acid	33.98		
3, 4, 5 cinnamic acid	4.63		

Table 4. Effect of red beetroot juices on body weight of rats.

Groups of rats	Initial weight (g)	Final weight (g)	Change in body weight (g)	Body weight gain (%)
G1	198 ^a ±2.33	234 ^a ±1.45	36.00 ^a ±2.08	18.18 ^a ±1.68
G2	195 ^a ±2.90	180 ^d ±2.96	-15.00 ^c ±1.33	-07.69 ^c ±0.25
G3	196 ^a ±1.76	228 ^b ±2.96	28.33 ^{ab} ±1.20	14.40 ^{bc} ± 0.65
G4	198 ^a ±3.05	221 ^c ±2.19	23.33 ^b ±3.88	11.25 ^{bc} ±0.98
G5	198 ^a ±2.96	226 ^b ±0.88	28.33 ^{ab} ±2.30	14.29 ^{bc} ±1.99
G6	199 ^a ±3.88	231 ^{ab} ±1.20	31.67 ^{ab} ±2.33	16.02 ^{ab} ±0.15

G1: Rats fed on basal diet (negative control),G2: Rats fed on anemic diet (positive control)

G3: Rats fed on anemic diet + ferrous sulfate (35 mg Fe/kg diet)

G4,G5,G6: Rats fed on anemic diet + red beetroot juice (5,10,15 ml/kg body weight/day)

Effect of red beetroot juice on Hemoglobin (Hb) and Hematocrit (HCT) levels of rats .

The results in Table (5) indicated that the Hemoglobin (Hb) and Hematocrit (HCT) levels was lower in anemic rats (G2) compared with those of control (G1) . The anemic groups fed on ferrous sulphate and red beetroot juice at all treatments had significantly higher serum hemoglobin (Hb) and Hematocrit (HCT) levels compared with anemic group (G2) .Oral administration of red beetroot juice improved the suppressive effect of anemic in groups 4,5 and 6 at feeding of 5,10 and15 mg/kg respectively. The amelioration by red beet root juice was occurred to the near normal values of control group. This means that red beetroot juice has protective effect .This may be due to that red beetroot juice improved levels of superoxide dismutase , Glutathione peroxidase , catalase enzymes and the presence of phytochemicals such as phenolic, flavonoids, Vit. E, Vit. C, β- Carotene ,thiamine .These results are in agreement with those of Maximas *et al.*, (2014).

Table 5. Effect of red beetroot juice on Hemoglobin (Hb) and Hematocrit(HCT) levels of rats .

Parameters	Hb (g/dL)	HCT (PCV %)
Group1	14.43±0.13 ^a	47.20±0.08 ^a
Group2	09.46±0.1 ^e	31.22±0.22 ^f
Group 3	13.45±0.11 ^b	44.26±0.16 ^d
Group 4	11.39±0.07 ^d	42.46±0.01 ^e
Group5	13.15±0.18 ^c	45.69±0.05 ^c
Group6	13.33±0.16 ^b	46.91±0.06 ^b

G1: Rats fed on basal diet (negative control),G2: Rats fed on anemic diet (positive control)

G3: Rats fed on anemic diet + ferrous sulfate (35 mg Fe/kg diet)

G4,G5,G6: Rats fed on anemic diet + red beetroot juice (5,10,15 ml/kg body weight/day)

Effect of red beetroot juice on red blood cells count (RBC) and white blood cell (WBC) levels of rats.

Feeding the rats on tannic acid caused a significant reduction in WBC and RBC counts as compared with control. Oral administration of red beetroot juice ameliorated the suppressive effect of tannic acid in group (4,5 and 6). The improvement occurred by feeding on red

beetroot juice is reported to the near normal values that were exhibited in control group. As shown in Table (6), The WBC count was raised by feeding on red beetroot juice nearly reached to Rats fed on basal diet (negative control), which was 9.11M/μL and groups fed on ferrous sulfate 8.49. The RBC count is raised feeding on red beetroot juice and reached nearly to the control group from 8.08M/μL These results are in accordance these off with Maximas et al. (2014).

Table 6. Effect of red beetroot juice on red blood cells count (RBC) and white blood cell (WBC) levels of rats.

Parameters	RBC (M/μL)	WBC (M/μL)
Group1	8.08±0.17 ^a	9.11±0.05 ^a
Group2	5.59±0.14 ^d	5.34±0.01 ^e
Group3	7.47±0.12 ^b	8.49±0.13 ^b
Group4	6.29±0.04 ^c	6.59±0.17 ^d
Group5	7.30±0.17 ^b	7.44±0.44 ^c
Group6	7.91±0.06 ^a	8.90±0.04 ^a

G1: Rats fed on basal diet (negative control),G2: Rats fed on anemic diet (positive control)

G3: Rats fed on anemic diet + ferrous sulfate (35 mg Fe/kg diet)

G4,G5,G6: Rats fed on anemic diet + red beetroot juice (5,10,15 ml/kg body weight/day)

Effect of red beetroot juice on mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) levels of rats.

The results in Table (7) showed that (MCV) and (MCHC) levels was lower in Rats fed on anemic diet (positive control) compared with those of Rats fed on basal diet (negative control). The anemic groups fed on ferrous sulphate and red beet root juice at all treatments had significantly higher serum mean corpuscular volume and mean corpuscular hemoglobin concentration levels compared with anemic group (G2). These results were accepted with Lotfi et al.,(2018).

Table 7. Effect of red beetroot juice on mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) levels of rats.

Parameters	MCV(fL)	MCHC(g/dL)
Group1	33.25±15 ^a	85.18±0.18 ^a
Group2	27.19±0.26 ^f	62.23±0.12 ^f
Group3	31.41±0.17 ^d	82.30±0.23 ^c
Group4	29.59±0.06 ^e	73.29±0.29 ^e
Group5	31.70±0.07 ^c	77.35±0.11 ^d
Group6	32.89±0.11 ^b	83.39±0.10 ^b

Finally, could be concluded that the feeding of rats on red beetroot juice increased Hb, HCT, RBC,WBC, MCV and MCHC values of serum iron profile subsequently red beetroot juice can be anti-anemic agent.

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تأثير تغذية الفئران المصابة بانيميا نقص الحديد على عصير الشمندر

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تهدف هذه الدراسة الى معرفة تأثير تغذية الفئران المصابة بفقر الدم الناجم عن نقص الحديد على عصير الشمندر الأحمر. أظهرت الدراسة الحالية أن الشمندر الأحمر يحتوي على مركبات لها القدرة على تحفيز امتصاص الحديد على وجه التحديد. تم تقدير التركيب الكيميائي للشمندر الأحمر ووجد انه غنى بالمعادن والبروتينات والألياف والكربوهيدرات والمركبات الفينولية والفلافونويد. أجريت تجربة التغذية على الفئران المصابة بفقر الدم الناجم عن نقص الحديد على عصير الشمندر. أشارت النتائج إلى أن نقص الحديد تسبب في الأثار السلبية التي تعكس الانخفاض الكبير في Hb و HCT و RBC و WBC و MCV و MCHC. على العكس من ذلك ، فإن تناول كلا من كبريتات الحديدوز وعصير الشمندر الأحمر تسبب في زيادة معنوية في قيم Hb و HCT و RBC و WBC و MCV و MCHC في مصل دم الفئران المصابة بالأنيميا.