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Beneficial Impact of Red Beetroots Powder on Iron Deficiency Anemic Rats

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ABSTRACT

This study was done to assess the impact of red beetroot powder alone or with sugarcane molasses on iron deficiency anemia that induced by iron depleted and high fiber diet (20 % Cellulose) in rats. Twenty-five female albino rats were separated into five groups: G1 fed on basal diet and served as a control group, G2 was fed on anemic diet and keep as anemic control group. G3 was fed on anemic diet with 1% iron sulphate. G4 and G5 were fed on diet group 3 with 10% red beetroots powder and 5% red beetroot powder with 5% sugarcane molasses, respectively. Duration of the evaluation was 30 days. Nutritional, biological and hematologic evaluations of tested rat groups were estimated. The findings indicated that a high fiber diet induced a notable reduction in the hemoglobin (Hb), hematocrit (HCT), and serum ferritin (SF) levels, while total iron-binding capacity (TIBC) was increased. Complete blood count was affected by high fiber diet.

Keywords: Iron deficiency anemia, red beetroots, and sugarcane molasses, rats.

1. Introduction

Iron deficiency is measured as one of the most predominant sorts of malnutrition. According to WHO (2011) iron deficiency is characterized by the absence of readily available iron reserves. Also it can define as plasma ferritin <12 µg/L that has not been adjusted for infection or inflammation.

Globally about 25% of people have anemia in 2021. Iron deficiency, is responsible for 50% of all anemia's incidence (Wawer and Jennings, 2018, Mantadakis *et al.,* 2020). The worldwide growing population in 15 years, has developed to become iron deficient with or without anemia and the number of people affected allegedly has escalated from 0.6 million to 3.5– 5 million (Stoltzfus, 2001). According to a study carried out in Egypt in 2010/2011, researchers reported that the occurrence of iron deficiency anemia was 25-30% in the overall population (Tawfik *et al.,* 2015). Iron deficiency anemia factors in under-development states may include not enough iron intake, low ingestion of iron enhancers (vitamin C, tocopherols and carotenoids) and/or high consumption of iron absorption inhibitors as legumes and cereals (Jbireal *et al.,* 2020).

Amongst vegetables, red beetroots (*Beta vulgaris L.*), versatile vegetable that is characterized by its reddish-purple color (Wruss *et al.,* 2015) and a remarkable source of vitamins B1 (thiamin), B2 (riboflavin), B3 (niacin), B6 (pyridoxine), folic acid, B12 (cyanocobalamin), ascorbic acid (vitamin C), nitrate, potassium, beta-alanine, sodium and magnesium.

Red beetroots is classified as a supper food due to its health benefits as it can treat anemia by raising blood cell count, enhancing blood flow and the ability of erythrocytes to transport oxygen, avoiding birth defects as it rich in folic acid and Vitamin B12 which is essential for erythropoiesis (El-Dreny *et al.,* 2019). The presence of ascorbic and citric acids in beetroot improves the absorption of iron in the body and aids its transportation in the intestines. Also red beetroots contains betalains which can be linked to iron ion transport in intestinal mucosa (Babarykin *et al.,* 2019). Also red beetroots can relax myocytes (smooth muscles), lowering high blood pressure through rising oxygen level, helps to boosting stamina via reducing oxygen during exercise, and the most significant point that it supports to recover anemia slowly and gradually naturally (El-Dreny *et al.,* 2019).

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Sugarcane Molasses may be utilized in the development of nutraceutical products to address iron deficiency anemia due to its content of iron, sulfur, fructose and copper. These components aid in the iron absorption, making it a potential dietary supplement for iron for individuals with iron deficiency anemia (Jain and Venkatasubramanian, 2017).

The goal of the current study was to examine ameliorative effect of red beetroots powder and its mixture with sugarcane molasses on iron deficiency anemia on rats.

2. Material and Methods

2.1. Materials

Red beetroots (*Beta vulgaris L.*), Sugarcane Molasses and oil were Picked up from the local market, Cairo, Egypt. All chemicals, reagents and Basal diet (AIN 93-G) ingredients including, cellulose, casein, starch, sucrose, minerals and vitamins mixtures were acquired from El-Gomhoriya Company Cairo, Egypt. Kits and Medical stuff were picked up from the Gamma Trade Co., for Pharmaceutical and Chemical, Dokki, Egypt. Rats, female albino rats total number 25 rats, Sprague Dawley strain, weighing (102-105 g) were picked up from National Research Center, Dokki, Egypt.

2.2. Methods

2.2.1. Preparation of red beetroots powder

Red beetroots were prepared according to the method of **Rahayu *et al.,* (2020)**, red beetroots were washed with tap water several times then sliced into thin slices and placed in an electric oven with fan at 45°C till dry. The slices were then ground to a powder and then placed in an air free bags.

2.2.2. Chemical evaluation of red beetroots powder

Moisture, ash, protein, fats, fiber were done using methods of AOAC (2005). Total carbohydrates were computed by difference using this formula:

Total carbohydrates % = 100- (% moisture + %crude protein + % fats+ % ash).

Minerals (Fe, Zn, Ca, Na and K) levels were assessed using AOAC (2005) method.

Red beetroots phenolic compounds levels were measured using HPLC analysis by Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector (Kim, *et al.,* 2006 and Mradu *et al.,* 2012).

2.3. Biological evaluation

2.3.1. Diets and experimental rats

Female albino rats (n=25), weighing 102-105g were used in this evaluation at animal house of National Research Centre. All animals were housed in cages and fed on a basal diet (American Institute of Nutrition, AIN-93). The composition of the basal diet was; casein (20%), sucrose (10 %), fats (10 %), minerals mixture (3.5 %), vitamins mixture (1 %), fibers (5 %) and starch (50.5 %) (Reeves *et al.,* 1993) for one week as adaptation periods.

Rats were divided into five groups, 1st group (5 rats) served as control group that fed on basal diet (AIN 93-G) and the remaining rats (20 rats) were fed on basal diet free of iron and high in callouses (20%) to induce iron deficiency anemia in 2 weeks till rats reached low blood Hemoglobin level compared with the control group (G1). Anemic rats, were divided into three groups, 2nd group was fed on diet free from iron and high in callouses 20 % all of the duration of the experiment and served as anemic rats group (G2), the 3rd group was fed on diet as G2 with 1% iron sulfate (G3), the 4th group was fed on diet as diet G3 with 10% red beetroot powder (G4) and 5th group was fed on diet as with 5% red beetroot powder and 5% sugarcane molasses (G5). Duration of the experiment was one month. Feed intakes were recorded and body weights were measured weekly. Treatments and animal maintenance were in accordance with the guidelines of the experimental animal house of Food Industry and Nutrition Institute, NRC Animal House and use committee, which follow the International Animal care and use Guidelines. After 4 weeks of the experiment, rats were fasted overnight, weighed, and blood samples were collected under mild diethyl ether anesthesia and plasma and serum were separated and stored at -20 °C for analysis. Rat organs including kidney, liver, heart, lungs and spleen were collected and weighted.

2.3.2. Biochemical investigation

Complete blood count was evaluated using automatic cell count using a hematological analyzer (Exigo Eos Vet, Sweden) following method of Walencik and Witeska., 2007. Blood Hemoglobin (Hb) and Hematocrit (HCT) levels of different rat groups were determined by the Cyano-methemoglobin method using method of Whitehead *et al.* (2019). Serum iron (SI) was measured according to Kok and Wild (1960), serum ferritin (SF) was determined following method of CDC (2016) and total iron-binding capacity (TIBC) were determined by Mahant *et al.* (2023). Malondialdehyde (MDA) was measured according to Lovric *et al.* (2008). Also, Transferrin saturation (TS) that mention to the proportion of the serum iron (SI) concentration to the TIBC, which is computed as described by Liu *et al.* (2013) equation:

$$TS(\%) = \frac{SI\left(\mu mol\ L^{-1}\right)}{TIBC\left(\mu mol\ L^{-1}\right)} \times 100\%$$

2.4. Statistical analysis

The data were expressed as mean ± SD, statistical analysis was done using SPSS statistical program, one-way ANOVA. Differences were considered remarkable at $p \leq 0.05$.

3. Results and Discussion

3.1. Chemical evaluation of red beetroots

3.1.1. Macro- and micronutrient contents of red beetroots

Some nutrients found in different levels in red beetroots and data is presented in table (1). Based on wet weight, the moisture represents the highest percentage 80%, followed by carbohydrates 15.8 % of macronutrients, while, potassium represents 25.2 %, calcium 9% and iron 0.6 % of micronutrients. So, red beet rot is a good source of these nutrients. El-Dreny and colleagues (2019) in their study provided the composition of red beetroots that contains high amount of moisture, protein, fats, ash and crude fiber and carbohydrate the values were 86.7%, 1.45 %, 0.38%, 1.53, 1.7% and 8.24%, respectively. Additionally, the authors detected slightly higher content of mineral composition, except sodium which was much higher with value 70.8 mg/100g wet weight. These variations in chemical constituents of red beetroots are valid as proven by Sawicki *et al.* (2016). Also, Ceclu and Nistor (2020) indicated that red beets are a low-fat vegetable and rich in carbohydrates, starch, soluble fibers, proteins. They confirmed that red beetroot contains some minerals such as potassium, sodium, iron, zinc, and calcium at levels close to our findings.

Table 1: Chemical composition of raw red beetroots.

Nutrients	Quantity (g/100 g fresh weight)
Moisture (g)	80
Ash (g)	1.4
Crude proteins (g)	1.2
Crude fats (g)	0.23
Crude fibers (g)	1.3
***Carbohydrates (g)**	15.8
Iron (mg)	0.6
Zinc (mg)	0.11
Calcium (mg)	9.0
Potassium (mg)	25.2
Sodium (mg)	5.3

3.1.2. Phenolic and Flavonoid Compounds of Red Beetroots

Red beetroots (*Beta vulgaris* L.) is one of the most powerful natural antioxidants vegetable source (Ceclu and Nistor, 2020). Some phenolic and flavonoid compounds of red beetroots are located in table (2), the data indicated that red beetroots have high levels of pyrogallol, gallic acid and benzoic acid, whereas hesperidin, catechin and catechol were the major flavonoids. Some studies done by El-Dreny *et al.* (2019), El-Mesallamy *et al.* (2020) and Arjeh *et al.* (2022) reported that red beetroots contain some phenolic and flavonoid compounds alike to the results in this study. Differences if present in

phenolic and flavonoid compounds may be due to the parts of the roots, species, variety, degree of maturity, harvest time and storage.

Table 2: Phenolic and Flavonoid Compounds (ppm) Content of Red Beetroots.

Compound	Quantity (ppm)
Pyrogallol	280.5
Gallic acid	15.2
Catechol	5.3
Catechin	25.2
Chlorogenic acid	6.3
benzoic acid	12
Ellagic acid	3.5
Hesperidin	200

3.2. Biological evaluation of red beetroots

3.2.1. Nutritional effects of red beetroots on anemic rats

As found in table (3), Rats that were fed on anemic diet (G2) displayed a considerable ($p<0.05$) decreases by 24.5% in body weight gain and 50% in feed efficiency ratio contrasted to rats that were fed on basal diet (G1). Results were close to what Susanti *et al.* (2017) found in their study. While, groups that were fed on 10% red beetroots or 5% red beetroots with 5% sugar cane molasses (G4 and G5) displayed considerable ($p\leq0.05$) increases in all nutritional parameters contrasted to anemic group (G2) and close to the basal diet group (G1). El-Dreny *et al.* (2019) indicated the same trend in their study with red beetroots juice.

Table 3: Nutritional values of different rat groups fed on tested diets.

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total feed intake (g)	Feed efficiency ratio
G1 (basal diet)	102.2 ±2.61	174.4±8.91^{b}	72.2 ±10.7^{a}	2000	0.04
G2 (iron free and high fiber)	103.3±10.01	145 ±4.82^{d}	40.5± 4.1^{c}	2600	0.02
G3 (G2 diet with1%$FeSO_4$)	104.5 ±3.24	157.8± 8.75^{c}	54.5±9.6^{b}	2550	0.02
G4 (G3 diet + 10% red beetroots powder)	101.8±7.19	175.8± 5.32^{b}	73.2±6.0^{a}	2450	0.03
G5 (G3 diet + 5% red beetroots powder+5% sugarcane molasses)	105.5 ± 0.2	180±0.7^{a}	74.5 ± 6.0^{a}	2500	0.03

The values in every column with distinct letters differ dramatically ($p\leq0.05$).

3.3. Biochemical evaluation

Complete blood test is mainly used to diagnose the iron deficiency anemia (Susanti *et al.,* 2017). As found in (Figure 1), blood levels of Hb, HCT, RBC, WBC, MCV, and MCHC were notably ($p\leq0.05$) lower in anemic rats (G2) than normal rats fed on basal diet (G1). These results were compatible with He *et al.* (2019), who applied the iron deficient diet on male Sprague-Dawley rats. Ali and Bilal (2023) detected the same changes in Hb, RBCs and SI in days 0, 7, 14, and 21 of the periodic study.

The Hb is the chief protein constituent in RBCs, which is responsible for delivering oxygen to various tissues of the body, in which iron placed in a central position in the Hb structure (Chen and Boyle, 2017). As shown in figure (1) the Hb value were less than 10 M cell/µl in anemic group (G2). If the iron dietary intake was insufficient to meet daily needs, the stored iron decrease to an insufficient level to sustain normal erythropoiesis. this induces iron deficiency anemia, followed by decrement in other blood indices such as HCT, MCV, and MCHC values as shown in current study data (Thakur *et al.,* 2019). The HCT is a measurement of the ratio of RBC volume to total blood volume (Carley, 2003). Since RBC count and HCT are directly related to Hb levels (Tang *et al.,* 2014), iron deficiency directed to a decrease in the Hb content, as shown with our findings. As shown in Figure 1, tested diets that including beetroot powder (G4, and G5) provided positive increments in all routine blood indices, in particular RBC and WBC counts as compared with anemic and $FeSO_4$ groups (G2 and G3). The RBCs count was raised in red beetroots powder groups G4 and G5. The values were 5.12 M/µL and 5.14 M/µL, respectively and almost reached the control group G1 values, which was 6 M/µL from 4.8 M/µL in anemic group (G2). These findings align with Maximas *et al.* (2014) and Hikmawanti *et al.* (2021).

Iron in circulation is necessary for the complete synthesis of Hb and RBCs (He *et al.,* 2019). Therefore, our findings confirmed that the diet supplemented with beetroots powder, as rich source of iron, could raise the blood Hb content and subsequently the RBCs counts, in particular when coupled with sugarcane molasses that is consider the most traditional source of iron among different consumers. The MCV mentions the average size of RBC, while MCHC reflects the Hb content of RBC (Tang *et al.,* 2014). Both MCV and MCHC are considered as average RBC indices (von Tempelhoff *et al.,* 2016). The findings in Figure (1) indicated that anemic rat group (G2) had lower levels of MCV and MCHC compared to rats fed on basal diet (G1). However, anemic groups fed with $FeSO_4$ and both concentrations of red beetroots powder (G3, G4, and G5) displayed considerably higher ($p \leq 0.05$) serum MCV and MCHC values when contrasted with anemic group (G2). These results were aligned with Lotfi *et al.,* (2018) and El-Dreny *et al.* (2019), who applied different volumes of beetroot juice to overcome dietary iron deficient anemia in female Soccer players and male albino rats. When iron deficiency occurred the Hb content in RBC was reduced, leading to a reduction in MCV and MCHC (Thakur *et al.,* 2019).

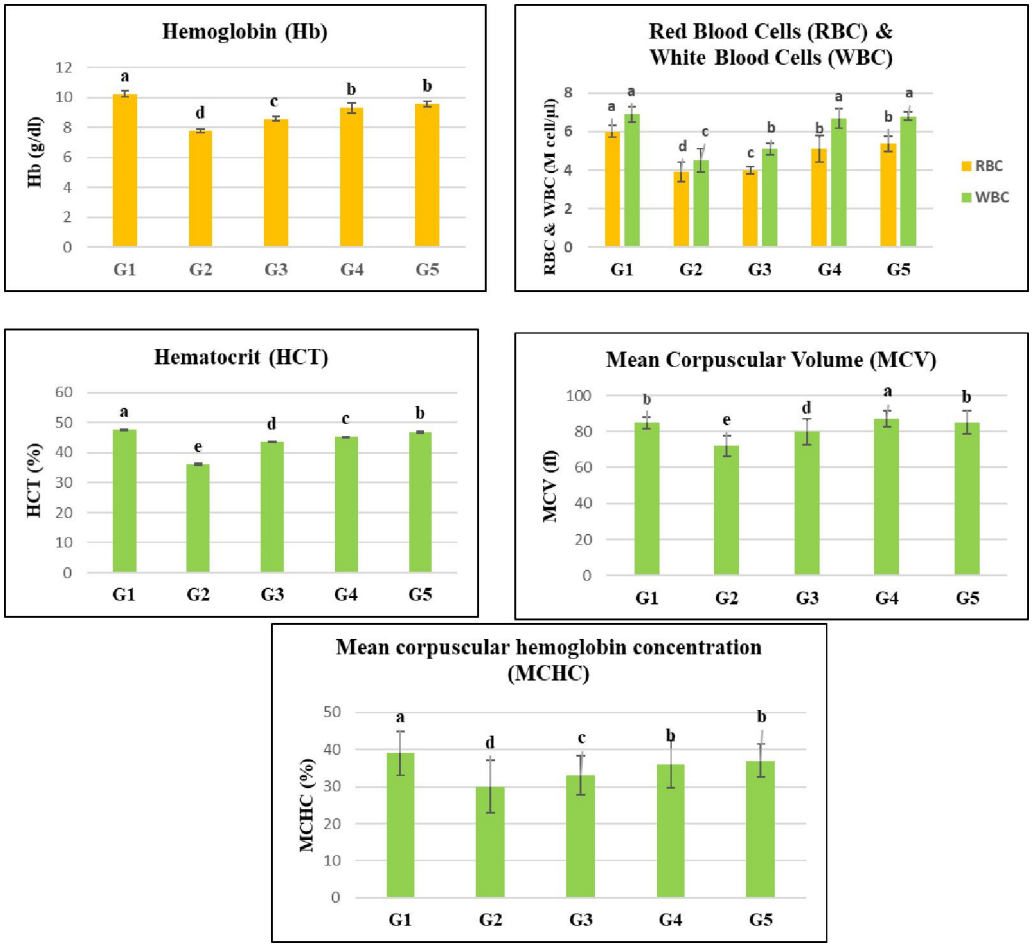

Fig. 1: Routine blood indices including Hb, HCT, RBC, WBC, MCV, and MCHC of anemic rat groups fed on tested diets.

G1= Basal diet , G2= Iron free basal diet, G3= Iron free basal diet +1%$FeSO_4$
G4= Iron free basal diet +1%FeSO4 + 5% beetroot + 5% sugarcane molasses,
G5= Iron free basal diet+1%FeSO4 + 10% beetroot
The values in every column with distinct letters differ dramatically ($p \leq 0.05$).

However, adding beetroots powder to the diet helped to restore these values to near-normal levels, indicating a full recovery of cell volume in anemic rats. Rats fed on red beetroots powder at a 5%

concentration coupled with 5% sugarcane molasses (G5) showed improvements in all blood indices comparable to rats fed on basal diet (G1). This is attributed to the antioxidant properties and existence of polyphenolic compounds, as well as antioxidant vitamins (vitamin C and E) in red beetroots (El-Dreny *et al.,* 2019). These findings are harmonious with those of Maximas *et al.* (2014) and Ali and Bilal (2023).

Figure 2 displays the content of MDA, the main marker of lipid peroxidation. In comparison with the rats fed basal diet (G1), the MDA value was considerably greater ($p<0.05$) in anemic rat serum (G2) by 22.7% increment. This data was in line with El Shemy (2018) and He at al. (2019) in rat model and with Smesim *et al.* (2024) in deficiency anemic Iraqi women. At the end of $FeSO_4$ and red beetroots treated groups (G3, G4, G5), the MDA value was considerably decreased ($p<0.05$) in all mentioned groups in contrast to the anemic group (G2). This was no significant difference in MDA values between the red beetroot 5% coupled with 5% molasses group (G5) and the normal group (G1), and also between the $FeSO_4$ group (G3) and the red beetroot 10% treated group (G4). Our findings were harmonious with Hikmawanti *et al.* (2021), who found the hydroethanolic extract of beetroot could potentially reduce the MDA increment in anemic rat model. The authors attributed that effect to the beetroot`s pigments and its betalains content. In particular, red beetroot 5% coupled with molasses 5% could be able to restore the antioxidant action in anemic rats to a normal state.

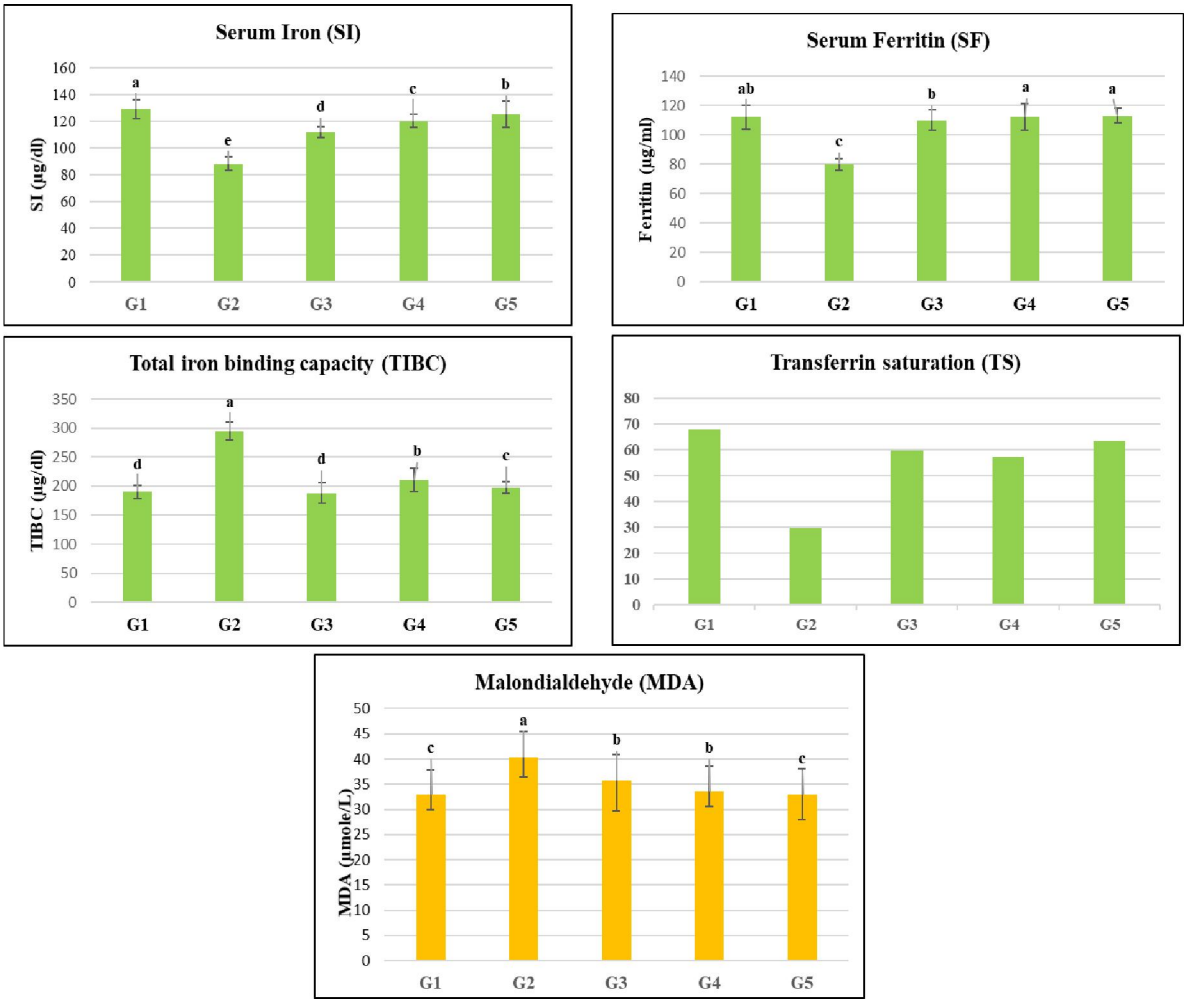

Fig. 2: SI, Ferritin, TIBC, TS and lipid peroxidation marker (MDA) of anemic rats fed on tested diet.
G1= Basal diet, G2= Iron free basal diet, G3= Iron free basal diet +1%$FeSO_4$
G4= Iron free basal diet +1%FeSO4 + 5% beetroot + 5% sugarcane molasses,
G5= Iron free basal diet+1%FeSO4 + 10% beetroot
The values in every column with distinct letters differ dramatically ($p\leq 0.05$).

The change trend in the MDA value was the opposite in the SI level in the anemic group (G2). The SI shows the total quantity of iron in the serum, while TIBC stands for the total capacity of iron that can bind to transferrin in the blood. Typically, TIBC and SI have an inverse relationship (He *et al.,* 2019). The TS measures the amount of iron bound to transferrin. The SF is a complex made up of apoferritin and ferric ion (Fe^{3+}), which can bind and store iron to maintain iron balance and Hb levels in the body. Therefore, the SF level is often considered a reliable indicator of the body`s iron reserves (He *et al.,* 2019).

The changes in iron profile parameters (SI, SF, TIBC and TS) are shown in figure 2. The data showed that all iron profile indices were considerably ($p<0.05$) lower, except TIBC notably higher in anemic group (G2) than normal diet group (G1). The increment of TIBC value in anemic group (G2) was due to progressive iron depletion from the body store as shown with SI content. These findings were consistent and expected with induced iron deficiency anemia model in rats from previous studies (Wang *et al.,* 2014, He *et al.,* 2019).

It is substantial to note that the levels of SI, SF and TS in the groups with added $FeSO_4$ and red beetroots powder (G3, G4 and G5) were significantly ($p < 0.05$) greater than those in the group (G2) and showed similar significance ($p > 0.05$) among themselves. Conversely, the TIBC levels in the $FeSO_4$, and beetroot added groups were considerably lower ($p < 0.05$) than those in the anemic group (G2). This notable decrease in TIBC might related to the iron improvement by red beetroot consumption (Al-aboud, 2018). This finding is consistence with Al-aboud (2018), who given red beetroot powder (8 g/d) to female subject for 21 days. One interesting observation was that beetroot had a greater impact than FeSO4, which nearly brought the SI, SF, TIBC and TS values back to normal. Moreover, adding beetroots powder (5%) coupled with sugarcane molasses (5%) was more effective in improving the routine blood stream indices, and raising the body`s level of transport iron.

4. Conclusion

This study concluded that red beetroots powder that used in this work enhanced the bioavailability of iron in anemic rats, adding 10 % of red beetroots powder was effective to cure iron deficiency anemia in rats. Red beetroots powder was effective when added to diet of 1% ferrous sulphate or combined with sugarcane molasses (1:1). Moreover, adding red beetroots powder at level 10- 5 % leaded to elevate rat's Hemoglobin (Hb) and serum iron percentage by 81% and 70 %, respectively when compare with anemic rats. This study recommends and confirmed the effective and important role of red beetroots in protective and cure iron deficiency anemia.

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