EFFICACY ASSESSMENT OF BEETROOT EXTRACT IN REGULATING IRON DEFICIENCY ANEMIA IN ANEMIC RATS

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ABSTRACT:

Objective: Comparison of beetroot extract and iron supplement (Iberet Folic) effects on iron and haemoglobin levels among iron deficient anaemic rats.

Methodology: In this study a natural source of iron, beetroot extract, and iron supplement (Iberet folic) was administrated to phenyl-hydrazine induced anaemic rats for 21 days. Beetroot was first dried, powdered, then extracted by ethanol.

Results: The beetroot in current experimental study showed improvements in haemoglobin, red blood cells, and iron serum as mean values of 23.8±0.69 g/dL, 15.16 ±0.54 106 /µL, and 31.5 ±1.5 µg/dL, respectively. As compared to beetroot, minor changes could be seen in mean values of haemoglobin, red blood cells, and serum iron of iron supplemented anaemic rats as; 22.4 ±0.443 g/dL, 13.69 ±0.5 106 /µL, and 30.1 ±0.9 µg/dL, correspondingly.

Conclusion: The Beetroot extract in this study presented as a viable replacement to the conventional local iron supplement.

Keywords: Beetroot, Beetroot extract, Anaemia, IDA.

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INTRODUCTION

Iron deficiency is measured as one of the most predominant sorts of malnutrition. Globally, on average, 50% of the anemia is presumed to be relatable to iron deficiency, and among the 26 risk factors, it ranks as number 9 and has cost 841,000 lives besides 35,057,000 disability-adjusted ones (Stoltzfus RJ. 2003). 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 RJ. 2001). 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 JM, *et al*., 2020). Amongst vegetables, beetroot is a remarkable source of nutrients as iron, nitrate, potassium, beta-alanine, sodium, folic acid, and magnesium. The advantages of consuming beetroot juice in human cases are, dealing with anemia, raising red blood cell numbers, increasing the oxygen-carrying capability of erythrocytes (RBCs), enlightening blood circulation, and make improvements to menstrual difficulties (Lotfi M, *et al*., 2019). On the other hand, a contrary impact of oral iron supplements negatively affects the treatment obedience and may lead to patients to withdraw from treatment. The most orally consumed iron supplements have been accompanied by epigastric distress, erosive mucosal injury in the upper gastrointestinal tract as well as vomiting, nausea, and other issues such as constipation and diarrhea (Cancelo-Hidalgo MJ, *et al*., 2013). Beetroot is a "Super Food" that contains vitamins B1 (thiamin), B2 (riboflavin), B3 (niacin), B6 (pyridoxine), B12 (cyanocobalamin) and ascorbic acid (vitamin C)., Being soluble nourishment with pH from 7.50 to 8.00. Beetroot has been fermented for its medicinal benefits and it prevents birth defects by folate, folic acid, also used as remedy for anemia in children, teenager, plus women. This low coast "Super Food" can be cooked/boiled, served as salad as well as its juice with any other fruits or root vegetables can be served as fresh juice. Furthermore, the medicinal benefits of beetroot may include relaxing of 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 (الدرینی اج, *et al*., 2019). Management of Iron Deficiency Anemia (IDA) in Conventional Medicine, without some side effects, is still a challenge; from limitation of the amount of consumption to secondary medicines, puts conventional medicines in a disadvantage against beetroot.

MATERIALS AND METHODS

Material Selection:

Inclusion Criteria: Fresh beetroots were procured from the market of Lahore.

Exclusion Criteria: Rotten beetroots and leaves were excluded from the study.

Subject selection:

Inclusion Criteria: Male rats having ages of 7 weeks and a weight of 180-200g were used as a biological model in the study (الدرینی اج, *et al*., 2019).

Exclusion Criteria: Diseased, underage and underweight rats were excluded from the Study (الدرینی اج, *et al*., 2019).

Methods:

Study design: Experimental (Rat bioassay).

Settings: Food Science and Technology Lab no. 101,102, Animal house of IMBB

University institute of Diet and Nutrition (UIDNS), The University of Lahore.

Duration of study: 9 months after approval of synopsis.

Sample size: 12 Male albino rats (4 rats in each group) were considered sufficient to depict the treatment effect and individual variation among test animals. It was because blood samples of each animal in the test and control group was separately analyzed (الدرینی اج, *et al*., 2019).

DATA COLLECTION PROCEDURE

Preparation of beetroot precipitates: Fresh beetroots were considerably washed to remove the grimes from their surfaces with distilled water. After washing the beetroots were sliced into thin pieces. This beetroot slices were dispersed in a tray to be dried absolutely at room temperature. Two weeks later the dried beetroot was collected and ground in the grinder to get a fine powder (Jaiswal A, *et al*., 2014).

Beetroot extract preparation: The Beetroot powder was subjected to hot percolation in a Soxhlet apparatus using ethanol. The extract was then dried and stored at 4°C till further use (Jaiswal A, *et al*., 2014).

Blood Sample Technique: During this experimental study, the rats observed fast for 12 hours before collecting blood sample in the morning (at day 0, 7th, 14th, and 21st), each rat was anesthetized by exposing to isoflurane to collect blood samples in tubes through cardiac puncture to check the response of treated diets on rats (الدرینی اج, *et al*., 2019).

DATA ANALYSIS PROCEDURE

1. Data was entered and subjected to statistical analysis using SPSS V. 25
2. Mean standard deviation was used for quantitative variables
3. Frequency and percentage were used for qualitative variables
4. The Mean differences of three types of extractions was analyzed by ANOVA
5. If the *p*-value comes out to be significant then the Tukey's-HSD test was applied for pair-wise means comparison.
6. The level of significance was taken as ($p \leq 0.05$)

RESULTS

Management of Iron Deficiency Anemia (IDA) in Conventional Medicine, without some side effects, is still a challenge; from limitation of the amount of consumption to secondary medicines, puts conventional medicines in a disadvantage against beetroot. Red beetroot is a natural super food. The therapeutic capabilities and other health benefits of beetroot juice which derived this research includes administration of iron deficiency anemia, disease fighting antioxidant potential, also it has a considerable quantity of vitamins B1, vitamin C, B2, niacin, B6, B12, and the leaves are also an excellent source of vitamin A. This research meant to analyze the consequences of beetroot extract comparing with local iron supplement Iberet Folic on iron and hemoglobin levels among iron deficient anemic rats. The beetroot was extracted by ethanol and administrated to the group of rats with basic diet and a group of rats with phenyl-hydrazine induced anemia. On the other hand, the third group of rats with induced anemia were treated with the iron supplement beside basal diet. The analyzed results in this study of all the considered parameters with discussion are summoned below:

Hemoglobin: Hemoglobin is one of the red blood cells protein accountable for the oxygen distribution to the tissues, in which the structure is highly dependent on iron. Iron deficiency anemia can cause Hb reduction, due to iron deficiency. The rats with normal hemoglobin were induced anemia by phenyl-hydrazine reducing the Hb level up to 30%. After 2nd day of anemia induction, the Hb level was recorded and for 21 days, one test group (anemic rats) was fed basal diet with beetroot extract (100ml/kg body weight) and the other one was treated with iron supplement (8mg Fe/kg body weight) plus basal diet. Following the interval of 21 days, mean values for the levels of Hb in the normal and anemic rats after treatment is presented in the Table 1 as following:

Groups		Study intervals (Hb Parameter g/dL)				
		Day 0	Day 7th	Day 14th	Day 21st	Mean
Healthy Group	**Basal diet group (BD)**	13.6 ±0.5	14 ±0.6	14.6 ±0.5	12.9 ±0.8	55.1 ±2.4
Anemic Test Group	**Beetroot extract group (BE)**	4.3 ±0.14	5.2 ±0.2	6.13 ±0.14	8.2 ±0.3	23.8±0.69
	Iron supplement group (IS)	4.34 ±0.2	4.98 ±0.07	5.99 ±0.08	7.09±0.143	22.4±0.443
	Means	22.24 ±0.84	24.18 ±0.87	26.72 ±0.72	28.19 ±1.2	

Table 1 Effect of Beetroot extract and Iron supplement on Hemoglobin level in anemic rats. BD = Basal diet + beetroot extract (100ml/kg body wt.); BE = Beetroot extract (100ml/kg body wt.) + Basal diet; IS= Iron supplement (8mg Fe/kg body wt.) + Basal diet. Values are expressed as means ± standard deviation.

Red Blood Cells: Beetroot is a natural source of iron, several other vitamins as well as antioxidants and phytochemicals. In contrast to the iron supplemented anemic rats' improvement in red blood cells can be observed as mean value 15.16 ±0.54 10^6/µL in the beetroot extract anemic group. Likewise, the normal rats with basal diet and beetroot extract also showed minor improvements in red blood cells before and after experiment during 21st days. Comparing the beetroot and iron supplement for treatment of iron deficiency anemia, beetroot extract consumed by anemic rats were discovered to be more effective than the iron supplement as shown in the Table 2 below:

Groups		Study intervals (RBCs Parameter 10^6/µL)				
		Day 0	Day 07th	Day 14th	Day 21st	Mean
Healthy Group	**Basal diet group (BD)**	6.8±0.6	7.4±0.4	8.08±0.18	8.61 ±0.36	30.9 ±1.6
Anemic Test Group	**Beetroot extract group (BE)**	2.2±0.13	2.98±0.198	3.95±0.099	6.03±0.114	15.16 ±0.54
	Iron supplement group (IS)	2.19±0.11	2.798±0.14	3.798±0.12	4.9 ±0.13	13.69 ±0.5
	Means	11.19±0.84	13.1±0.74	15.83 ±0.41	19.09 ±0.6	

Table 2 Effect of Beetroot extract and Iron supplement on red blood cells level in anemic rats. BD = Basal diet + beetroot extract (100ml/kg body wt.); BE = Beetroot extract (100ml/kg body wt.) + Basal diet; IS = Iron supplement (8mg Fe/kg body wt.) + Basal diet. Values are expressed as means ± standard deviation.

Serum Iron: Serum iron is a measurement of circulating amount of iron in blood which bounds to serum ferritin plus transferrin and it is a medical laboratory test for detecting iron deficiency anemia. Beetroot, the wonder vegetable, contains iron and vitamin C which boost iron absorption. Comparing the effect of beetroot extraction and iron supplement on serum iron among anemic rats, we found the natural source of iron; beetroot had higher mean value of 31.5 ±1.5 µg/dL than iron supplement with mean value of 30.1 ±0.9 µg/dL. Also, the rats with standard diet and normal health were fed beetroot where the minor increase in serum iron can be observed in the given Table 3 below as following:

Groups		Study intervals (Serum Iron Parameter µg/dL)				
		Day 0	Day 07th	Day 14th	Day 21st	Mean
Healthy Group	**Basal diet group (BD)**	96.58 ±5.3	96.59 ±5.5	97.2 ±5.55	98.01 ±5.6	97.1 ±5.49
Anemic Test Group	**Beetroot extract group (BE)**	30.96 ±1.34	31.43 ±1.5	31.99 ±1.6	32.67 ±1.57	31.5 ±1.5
	Iron supplement group (IS)	29.43 ±0.7	29.63 ±0.8	29.93 ±1.1	31.5 ±1.06	30.1 ±0.9
	Means	156.97 ±7.34	157.6 ±7.8	159.1 ±8.3	162.2 ±8.23	

Table 3 Effect of Beetroot extract and Iron supplement on Serum Iron level in anemic rats. BD = Basal diet + beetroot extract (100ml/kg body wt.); BE = Beetroot extract (100ml/kg body wt.) + Basal diet; IS = Iron supplement (8mg Fe/kg body wt.) + Basal diet. Values are expressed as means ± standard deviation.

DISCUSSION

The purpose of our presented research was to compare the effect of natural source of iron Beetroot extract and the medicinal iron supplement that which source is more capable for iron deficiency anemia among anemic rats. The results of this study of proximate analysis were more considerable as compared to the earlier studies. Beetroot can increase hemoglobin and red blood cells levels as evidenced by the study of El-Dreny *et al*, Favour. Nyoh Beshel *et al*, Anupam Jaiswal *et al*, and Mohy Eldin Abd-El-Fattah[6,10,11,15] investigated the sample of Beetroot juice or Beetroot extract and reported notable increasing values as 13.33 ±0.16 g/dL, 10.26

±0.07 g/dL, $p < 0.01$, 15.3 ± 0.35 g/dL, 13.45 ±0.11 M/µL, 5.05 ±0.19 10^6/µL, $p < 0.001$, and 6.7 ± 0.25 10^6/µL respectively, also the effects of iron supplement on hemoglobin and RBCs are observed by Mohy Eldin Abd-El-Fattah *et al* as following; 14.4 ±0.40 g/dL and 6.7 a ±0.25 10^6/µL[15]. Another experimental study by Thakur MK *et al* reported impact of commercial available iron deficient and normal food against anemic rats and significant difference was observed in anemic rats' hemoglobin, RBCs, and serum iron (SI) levels as 7.5 ±0.3 g/dL, 4.8 ±0.2 10^6/µL, and 76.8 ±1.4 µg/dl[34]. Studies conducted earlier provided with the prove that the results of our study was coordinating with the studies held before. The grand means for the levels of hemoglobin in the test groups BE and IS were found to be 23.8 ±0.69 g/dL and 22.4 ±0.443 g/dL, respectively. While the means for the intervals of 0, 7th, 14th, and 21st days were noted to be, 22.24 ±0.84 g/dL, 24.18 ±0.87, 26.72 ±0.72 g/dL, and 28.19 ±1.2 g/dL, correspondingly. Comparing mean values of BE and IS, the beetroot extract BE had better impact on hemoglobin level (mean value of BE 23.8 ±0.69 g/dL) than IS with minor change as 22.4 ±0.44 g/dL. On the other hand, the rats with normal hemoglobin treated with basal diet and beetroot extract showed significant increase in hemoglobin level respectively. As shown in Table 02, the red blood cells concentration in anemic rats (test group BE) after the 21st day of observation, had significantly increased during administration of beetroot extract other than iron supplemented anemic rats (test group IS), with a mean RBC count of BE and IS as 15.16 ±0.54 10^6/µL, 13.69 ±0.5 10^6/µL, respectively. Also notable improvement in RBCs level (mean value: 30.9 ±1.6) of normal rats were seen after administration of beetroot extract beside basal diet. The means for the intervals of 0, 7th, 14th, and 21st days were observed to be as following; 11.19 ±0.84 10^6/µL, 13.1 ±0.74 10^6/µL, 15.83 ±0.41 10^6/µL, and 19.09 10^6/µL ±0.6 10^6/µL, respectively.

After ethical considerations, the rats were injected phenylhydrazine (60 mg/kg) for 3 days (20 mg/day) inducing iron deficiency anemia. The data collected showed significant decrease in hemoglobin and RBCs concentration which was also an indication to low serum iron in anemic rats. Serum iron is a measurement of circulating amount of iron in blood and SI test is run to examine serum iron. In our study the Weiner Lab (Argentina) serum iron kit was used to calculate the serum iron (SI) after 21st days of beetroot extract and iron supplement administration. The rats observed fast for 12 hours before collecting blood sample in the morning (at each day 0, 7th, 14th, and 21st). The results of our study (Table 03) were in line of the data established by Thakur MK *et al*, in the study of them iron was eliminated from diet for 35 days to induce iron deficiency anemia in rats. After iron withdrawal, lower levels of serum iron (77 ±2.5 µg/dL) were observed as compare to standard diet of control group (114.8 ±5.4 µg/dL)[34]. As presented in Table 03 the significant difference can be seen comparing results of beetroot extraction and iron supplement on serum iron level. The healthy rats fed on standard diet and beetroot extract showing an increased mean value from 96.58 ±5.3 µg/dL to 97.1 ±5.49 µg/dL, correspondingly, the beetroot extract presented 31.5 ±1.5 µg/dL greater mean value than iron supplemented 30.1 ±0.9 µg/dL group of anemic rats. The grand mean values for interval of 21st days of (0, 7th, 14th, 21st) as following; 156.97 ±7.34 µg/dL, 157.6 ±7.8 µg/dL, 159.1 ±8.3 µg/dL, 162.2 ±8.23 µg/dL, respectively.

Conclusion: Beta vulgaris also known as Beetroot has an exclusive nutritious component that remarkably add to its usage as a functional super food. Beetroot is a rich natural source of phytochemicals, certain vitamins and minerals such as iron (Fe), calcium (Ca), sodium (Na), copper (Cu), potassium (K), manganese (Mn), and zinc (Zn) which are most likely functional ingredients accountable for its hematinic outcomes. The present study was conducted in order to investigate the effect of beetroot extract comparing the local iron supplement against iron deficiency anemia. Three different groups of rats were treated with beetroot extract and iron supplement beside basal diet. Observing the results of this analysis we examined that the beetroot extraction had greater and significant improvements in hemoglobin, RBCs, and iron concentration as compare to iron supplement. This study provides us the solid proves that beetroot can be a powerful natural source of iron, vitamins, and phytochemicals enhancing the recovery of iron deficiency anemia in human as well. This is because rats have same genetic makeup as of the human so to take this study further to human could prove worthy. Beetroot can also be used in foods and consumed on daily basis as salad and additives (coloring food agent in powder form) for its health outcomes.

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