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Screening of different doses of lyophilized beetroot for their hematopoietic potential.

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Abstract

Background: Beetroots are considered a vital source of nutrition and are usually incorporated into the daily diet to prevent different disease conditions. The therapeutic effect observed is due to the presence of active ingredients in the vegetables. The majority of our population, especially females, suffers from anemia due to malnutrition. The perseverance of this research was to explore the hematopoietic effects of lyophilized beetroot powder at different doses so they could be recommended as a nutritional supplement.

Methodology: Albino rabbits weighing 1500-2000 gm of either gender were selected for the study and alienated into three sets. Set I was administered distilled water, whereas sets II and III were given lyophilized beetroot 500 mg/kg and 1000 mg/kg, correspondingly. For hematopoietic evaluation of blood, automated huma count plus was utilized. After adjusting according to their weights, lyophilized beetroot powder was suspended in distilled water and given at 500 mg/kg and 1000 mg/kg to rabbits. They were dosed once daily for two months.

Results: The effects were verified before the dosing was scheduled and on the 30th day and 60th day of dosing. The outcomes presented a highly noteworthy increase ($p<0.0001$) in hemoglobin, erythrocyte count, hematocrit, leukocyte count, and thrombocytes by both doses. 500 mg/kg dose showed a more significant increase in leukocytes and thrombocyte count compared to 1000 mg/kg, whereas 1000 mg/kg showed a more substantial increase in erythrocytes and Hb as compared to 500 mg/kg at the conclusion of the study period.

Conclusion: From the above, it can be concluded that beetroot possesses pronounced hematopoietic effects and might be beneficial as a non-pharmacological treatment for anemia and thrombocytopenia.

Keywords

Beetroot, Erythrocytes, Hemoglobin, Thrombocytes, Leukocytes.

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Introduction

Fruits and vegetables are considered essential dietary nutrients that should be consumed by humans daily. Not only are they healthy and contain different macro and micronutrients, but they are also good sources of anti-oxidants[1]. The population of Pakistan, particularly the female gender, is mostly anemic[2] due to many factors in which diet plays a fundamental and essential role. A pregnant female's diet is mainly compromised, which ultimately leads to anemia in newborn infants too. Dietary sources such as dried apricot, dried dates, and blackstrap molasses have been considered excellent sources of iron, nearly similar to ferrous sulfate[3]. Garden beet or *Beta vulgaris* belongs to the family Amaranthaceae and is usually found in the different parts of Asia, near the East and Mediterranean coastline[4].

Beta vulgaris is rich in many minerals and vitamins. According to the dictionary of Oxford food and science, beetroot is also a high source of iron, and around 100 gm of beetroot contain 0.8 milligrams of iron[5]. It plays an essential role in gaining and maintaining healthy hemoglobin in humans[6]. *Beta vulgaris* contains many nutrients that simultaneously help maintain health, such as flavonoids, carotenoids, tannin, alkaloids, phenols, and vitamins C, B_3, B_6, and B_9. These constituents help in the absorption of iron in the gut[7].

Anemia is a severe pathological condition characterized by insufficiency of red blood cells or hemoglobin in the blood. People having anemia might experience fatigue, breathlessness, brittle nails, pale skin, headaches, tongue soreness called glossitis, cracking in oral mucosa, and susceptibility to infections. There are many types of anemia, but Iron deficiency anemia is the most common type of anemia, which can easily be overcome by taking fruits and vegetables rich in iron[8].

In females, the anemic condition usually occurs in pregnancy when there is high plasma volume compared to red mass cells. This is also known as physiological anemia, and to treat the condition, doctors usually prescribe a healthy diet full of iron[9]. There are many other causes of anemia like malnutrition, malabsorption, and blood loss due to any pathological condition.

Beta vulgaris, due to its high iron content, can be used in many such conditions. Traditionally, it was used in pulmonary disorders, cardiovascular diseases, blood disorders, and gastrointestinal problems. Ancient people also used this vegetable for cosmetic purposes, anti-inflammatory[6], anti-nociceptive[10], nephroprotective[11], and diuretic[12]. It has also been reported that *Beta vulgaris* is a helpful product for the treatment of infertility and reproductive disorders[13] and has positive effects on thyroid hormones[14]. Besides that, literature studies have shown the efficacy of beetroot juice in treating hypertension due to the presence of nitrates[15]. The present study was conducted to see the effect of beetroot as a hematopoietic agent at different doses.

Methodology

Lyophilized Beetroot Powder

The lyophilized powder of beetroot was acquired from the group Sun Rise Nutra Chem. The Lot number of beetroot lyophilized powder was Ctc 2015 0320. For the safest packaging and storing of the powder, a zip-lock plastic bag was used. Furthermore, aluminum foil was used as the powder was photosensitive. The purity of the powder was assured by the vendor and again verified by getting the powder evaluated by the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi.

Animal Selection

For the present study, albino rabbits weighing 1500-2000 gm of either gender were selected and were divided into three groups comprising n=10 respectively. The first group was given distilled water as a control, whereas Groups II and III were given 500 mg/kg and 1000 mg/kg lyophilized beetroot orally for 60 days. The animals were kept in the animal house of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi and were given food and water ad libitum.

The animals were handled as per the Helsinki Resolution 1964 guidelines, and the study was authorized by the Institutional Board of Advanced Studies and Research vide Resol. No. 10 (P) 18 University of Karachi.

Gathering of Sample

The collection of samples was done by cardiac rupture of rabbits on the baseline, then on the 30th day, and the 60th day[16]. The required sample for the hematology study of 2 ml was taken in an EDTA (Ethylene diamine tetraacetic acid) K3 tube. Post cardiac puncture, the animals were observed for restlessness and other symptoms associated with hemodynamic instability.

Procedure

For the separation of blood plasma, centrifugation of blood sample was accomplished using Humax 14 K (Germany) for 15 minutes at 3000 revolutions per minute (RMP). The instrument used to determine Erythrocytes, leukocytes, and Thrombocyte count was Huma Count plus analyzer (Model # 6400/ S) by Human Germany. Hemoglobin and hematocrit levels were also determined[17].

Statistical Analysis

Analysis of statistics was carried out by captivating means of all the values of treated animals associated with means and standard deviation of all the groups by applying the formula of two ways ANOVA (analysis of variance). The post hoc Turkeys test was used for multiple comparisons. SPSS version 21.0 was used for analysis. The p-value $p \leq 0.05$ was considered significant.

Results

The effect of beetroot at different doses on erythrocytes, hemoglobin, and hematocrit (Hct) has been shown in table 1. The evaluations were recorded at baseline and 30 and 60 days of treatment. Both beetroot doses reported an extremely substantial ($p \leq 0.001$) increase in hemoglobin level, erythrocyte count, and hematocrit levels on the 30th day of dosing. The hemoglobin level was significantly increased by both doses on the 60th day too. However, the erythrocyte count and hematocrit were elevated by 1000 mg/kg dose only compared to control.

Beetroot 1000 mg/kg significantly increased Hb concentration, erythrocyte count, and Hct concentration on the 60th day compared to the low dose.

Table 1: Impact of various doses of beetroot on hematological activity.

Groups	Days	Hb (gm/dl)	Erythrocytes (million/µl)	Hematocrit (%)
Control	Baseline	8.9±0.26	4.3±0.18	30.3±1.25
	30 days	8.7±0.39	4.32±0.3	31.7±1.63
	60 days	8.6±0.53	4.34±0.23	32.5±1.9
***Beta vulgaris* 500 mg/kg**	Baseline	8.63±0.34	4.2±0.25	31.8±1.39
	30days	10.6±0.21***	5.16±0.14***	35.9±0.87***
	60 days	9.6±0.87***	4.16±0.10*	28.3±0.68***
***Beta vulgaris* 1000 mg/kg**	Baseline	8.7±0.18	4.19±0.10	31.7±1.63
	30 days	10.15±0.16***!!!	5.21±0.12***	33.9±0.48***!!!
	60 days	10.6±0.15***!!!	4.96±0.57***!!!	33.5±0.30!!!

*p≤0.05, **p≤0.01, ***p≤0.001 = significant, very significant, and vastly significant as associated to control.
!p≤0.05, !!p≤0.01, !!!p≤0.001 = significant, very significant and vastly significant, when associated among treated groups.

Table 2 revealed the outcome of various doses of beetroot on Mean corpuscular hemoglobin (MCH), Mean corpuscular volume (MCV), and Mean corpuscular hemoglobin concentration (MCHC) after 60 days of dosing. The observations were noted at baseline and on the 30th and 60th days of treatment. Analysis of Post hoc by

Tukey's test revealed a highly remarkable (p≤0.001) rise in MCH, MCV, and MCHC on the 60th day by 500 mg/kg dose of beetroot compared to control. Beetroot 1000 mg/kg extremely substantially (p≤0.001) declined MCH, and MCV throughout the treatment period compared to control. However, an extremely substantial (p≤0.001) rise in MCHC was detected by 1000 mg/kg dose on the 60th day of dosing.

Comparing treated groups indicated a substantial (p≤0.001) decline in MCH, MCV, and MCHC by 1000 mg/kg of beetroot when paralleled with 500 mg/kg during the treated period.

Table 2: Impact of various doses of Beetroot on Blood Parameters

Groups	Days	MCH (pg)	MCV (fl)	MHC (gm/dl)
Control	Baseline	20.3±0.25	65.3±0.18	30.56±0.61
	30 days	20.5±0.35	65.1±0.17	30.07±0.71
	60 days	20.6±0.24	65.3±0.14	30.7±0.19
***Beta vulgaris* 500 mg/kg**	Baseline	20.5±0.19	65.2±0.94	30.67±0.27
	30 days	20.4±0.13	67.7±0.28***	29.7±0.15*
	60 days	22.0±0.49***	68.8±0.15***	31.8±0.14***
***Beta vulgaris* 1000 mg/kg**	Baseline	20.0±0.96	65.2±0.16	30.31±0.53
	30 days	19.04±0.11***!!!	64.2±0.16***!!!	30.02±0.28
	60 days	19.5±0.45***!!!	61.3±0.28***!!!	31.4±0.17***!!

*p≤0.05, **p≤0.01, ***p≤0.001 = significant, very significant, and vastly significant as associated to control.
!p≤0.05, !!p≤0.01, !!!p≤0.001 = significant, very noteworthy and vastly substantial, when related among treated groups.

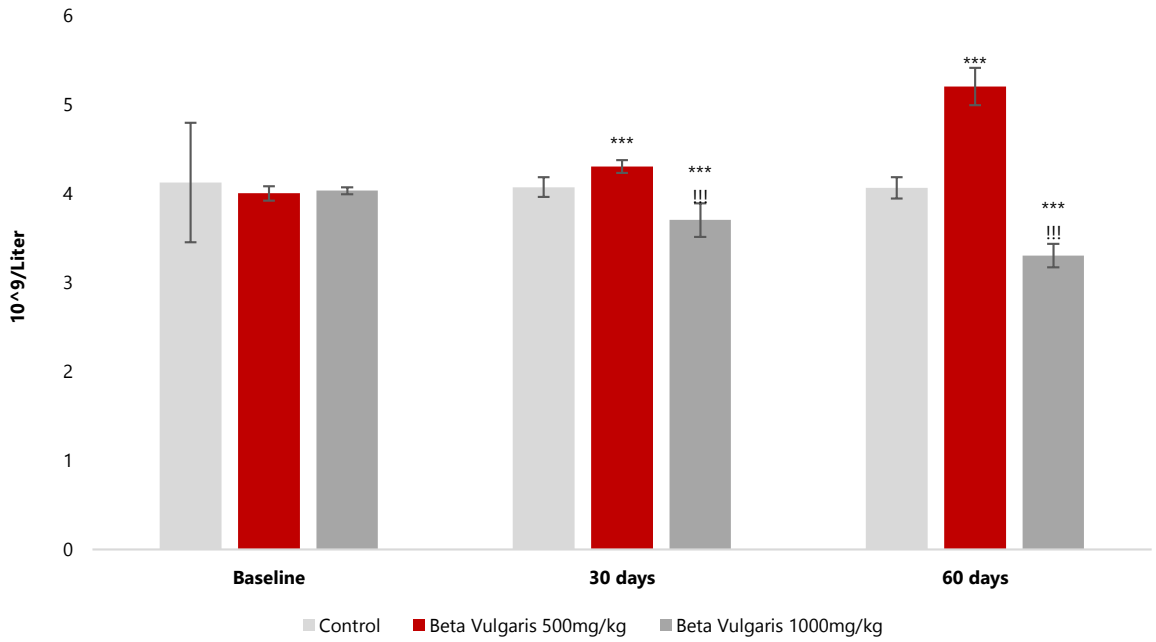

Figure 1: Impact of various doses of Beetroot on Leukocyte Count.

*p≤0.05, **p≤0.01, ***p≤0.001 = significant, very significant, and vastly significant as associated to control.
!p≤0.05, !!p≤0.01, !!!p≤0.001 = significant, very significant and vastly significant, when associated among treated groups.

Figure1 showed the influence of various doses of beetroot on leukocytes posts 60th day dosing. Analysis of post hoc by Tukey's test showed extremely considerable (p≤0.001) escalation in leukocyte count by 500 mg/kg dose of beetroot during the treatment period compared to control. However, there was a substantial (p≤0.001) decline in leukocyte count by 1000 mg/kg dose of beetroot during the course of the treatment time related to control. The contrast between treated groups showed a particularly substantial (p≤0.001) decline in leukocyte count by 1000 mg/kg dose of beetroot linked to 500 mg/kg throughout the treatment period.

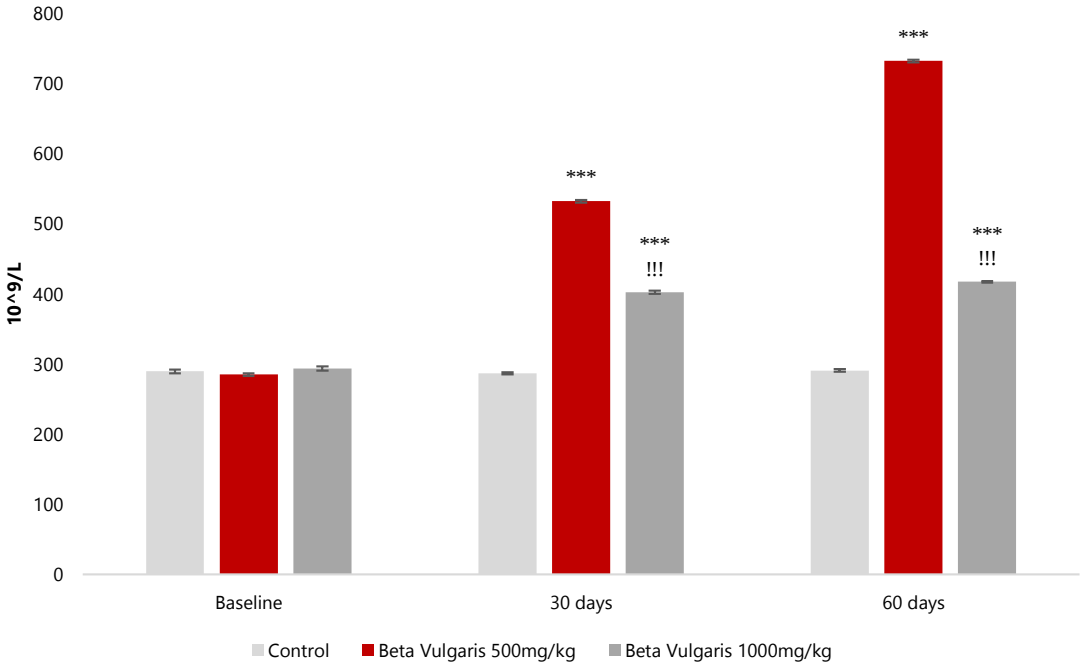

Figure 2: Impact of various doses of beetroot on Thrombocytes.

*p≤0.05, **p≤0.01, ***p≤0.001 = significant, very significant, and vastly significant as associated to control.
!p≤0.05, !!p≤0.01, !!!p≤0.001 = significant, very significant and vastly significant, when associated among treated groups.

Figure 2 showed the effect of beetroot at various doses on thrombocyte count after 60 days of dosing. The interpretations were detected before treatment and at 30 and 60 days of management. The test analysis by Tukey's test demonstrated an extremely substantial increase (p≤0.001) in thrombocyte count by beetroot of both the doses during the treatment period compared to control. Throughout the treatment period, the 1000 mg/kg dose of beetroot resulted in a substantial (p≤0.001) drop in thrombocyte count when compared to the 500 mg/kg dose.

Discussion

Beta vulgaris has been traditionally reported to play an essential role in improving blood parameters. Our results showed remarkable and affirmative effects of both doses on hematological parameters. *Beta vulgaris* (100 gm) contains 109 µg folate, 4.9 mg vitamin C, and 0.8 mg iron[18]. Hemoglobin present in red blood cells is a metalloprotein protein. It is vital for transporting oxygen in the body and having other significant functions, and hemoglobin deficiency due to physiological or pathological conditions cause anemia. *Beta vulgaris* has main constituents, such as folic acid, iron, and vitamin C play a vital role in treating and managing anemia. Folic acid is also an essential water-soluble vitamin, and its deficiency causes megaloblastic anemia due to macrocytosis of erythrocytes. Literature studies carried out on the anti-anemic effects of ethanolic extract of 200 mg/kg of beetroot have revealed positive effects[19].

It was also revealed in recent research that beetroot juice showed significant improvement in hemoglobin values in iron-deficient adolescent girls[20]. A well-known researcher at Sao Paulo's University Department of Pediatrics recommended that humans suffering from malnutrition due to low socioeconomic status can be given an iron-rich diet at home. Beets were the significant constituents of that diet which had given effective results, especially in the female population[21]. Yet all literature studies have shown that beets should be delivered in combination with other supplements rich in iron to achieve better results. The presence of iron, folate and vitamin C in beetroot helps produce erythrocytes, ultimately increasing hemoglobin levels.

Leukocytes are an essential part of the immune system, which have many functions. One of the primary functions is to protect from the invasion of foreign organisms[22]. Doctors generally observe the process of wound healing through leukocyte count[23]. The result of our study revealed an increase in leukocyte count at 500 mg/kg dose throughout the study; however, 1000 mg/kg showed a decrease in leukocyte count, as shown in figure 1. Researchers have reported that sugar and vitamin C have a similar structure in terms of molecules, so sugar molecule competes with vitamin C, which leads to less ingress of vitamin C in leukocytes, which leads to reduced production of leukocytes[24]. Our study indicated that 1000 mg/kg had less concentration of leukocytes because of high levels of sugar in beetroot, which competes with vitamin C and causes low levels of leukocytes.

One more important constituent of blood is Thrombocytes which play a significant part in maintaining hemostasis[25]. Bleeding risk increases if platelets count is low and if the count of platelets increases, it causes thrombosis. Our study showed an increase in the count of thrombocytes by both doses, which were in the normal reference range. This is because beetroot contains vitamin C, which prevents free radical destruction of thrombocytes and helps in increasing its number[26]. Beetroot contains nitrates; literature studies have reported that platelet-derived nitrous oxide and endothelium-derived nitrous oxide (NO) prevent fibrin formation and platelet aggregation, thus inhibiting the generation of thrombus[27].

The limitation of the study is that it is a preliminary study depicting the effect of beetroot on hematological parameters at different doses. Based on these results, it is recommended to develop further models of anemia, leukemia, and different cardiovascular disorders to evaluate their effect on a diseased model and later on humans.

Conclusion

From our study, we concluded that beetroot possesses hematopoietic effects at both 500mg/kg and 1000 mg/kg doses. Anemia is one of the most prevailing disease conditions in the female population of Pakistani society. The use of beetroot as a nutritional supplement would be beneficial as it would increase the Hb and erythrocytes without causing any adverse effects. Besides that, thrombocytopenia occurs in many disease conditions. This powder would be beneficial in increasing the platelet count too. Further studies need to be conducted to evaluate the exact molecular mechanism responsible for these actions.

Conflicts of Interest

The authors have declared that no competing interests exist.

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