Protective Effects of Beetroot Extract against Phenyl Hydrazine Induced Anemia in Rats

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ABSTRACT

Background: Anemia can be regarded as one of the oldest blood malformation known over a century. Megaloblastic anemia arises due to curtailed formation of erythrocytes, which leads to formation of a large number of underdeveloped erythrocytes. Proper nutritional supplementation may be useful in such condition. *Beta vulgaris* or beetroot is one of the important vegetables consumed worldwide. The aim of the present work was to evaluate anti-anemic potential of beetroot. **Methods:** Beetroot was extracted with ethanol. Phytochemical and phytoanalytical studies were performed on extract. Anemia was induced by phenyl-hydrazine. Animals were treated with extract throughout the study for 24 days. The red blood cell (RBC) number and hemoglobin concentration were determined every 3 days for 24 days. **Results:** Extract was found to be rich in folic acid, ascorbic acid, and iron. Following the induction of anemia, the number of erythrocytes and the hemoglobin concentration decreased by 62.51% and 69.64%, respectively. Administration of standard hematinic preparation and extract (200 mg/kg) resulted in significant increase ($P < 0.001$; $P < 0.01$) in the number of RBCs as well as hemoglobin concentration when compared to the untreated phenyl hydrazine-induced anemic rats. **Conclusion:** Extract effectively raised the level of hemoglobin and erythrocyte count at dose 200 mg/kg. Vitamin and minerals found in beetroot are most likely active ingredients responsible for its hematinic effects. Still, methodical studies are obligatory to derive its effects on humans.

Keywords: *Beta vulgaris*, erythrocytes, hemoglobin, phenyl hydrazine

INTRODUCTION

Anemia can be regarded as one of the oldest blood malformation known over a century.[1] It is a disorder of blood cells characterized by manifestation of giant erythrocytes. Megaloblastic anemia arises due to curtailed formation of erythrocytes, which leads to formation of a large number of underdeveloped erythrocytes. Such erythrocytes cannot function like normal one; such cells also have short lifespan. Folic acid deficiency could be considered as a major cause of this malformation. Proper nutritional supplementation may be useful in such condition.

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DOI: *10.5530/pj.2014.5.1*

Beta vulgaris or beetroot is one of the important vegetable consumed worldwide. Scientifically, beetroot is recognized to increase exercise stamina[2] and increases running performance.[3] it is also utilized in management of hypertension.[4,5] It is recognized for antiradical, antimicrobial, and cytotoxic activities.[6,7] It is also known for hepatoprotective[8] and antidiabetic[9] potential.

Due to red in color, traditionally, beetroot is claimed to be useful in hematological disorders. However, no scientific evidence is available pertaining to it. Hence, the aim of the present work was to evaluate anti-anemic potential of beetroot.

EXPERIMENTAL

Plant collection

Beetroot *B. vulgaris* was collected from local market of Jabalpur in the month of March 2013. It was then identified and authenticated by Dr. A.B. Tiwari, Sr. Scientist,

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Extraction

B. vulgaris were washed sliced and shade dried under room temperature for a period of 2 weeks. The dried plant material was powdered and 100 g of powder was subjected to hot percolation in a soxhlet apparatus using ethanol. Extract was then dried and stored at 4°C till further use.

Phytochemical and phytoanalytical studies

Phytochemical tests

The extract was subjected to phytochemical analysis as per reported method.[10]

Estimation of folic acid

The standard or sample solution of folic acid 1.0 ml were mixed with 1.0 ml of 4 mol L-1 hydrochloric acid, 1.0 ml of 1% (w/v) sodium nitrite, 1.0 ml of 1% (w/v) sulfamic acid and 1.0 ml of 1% (w/v) 3-aminophenol, which was resulting in an orange-yellow complex. The absorption of complexation was measured at 460 nm using ultraviolet (UV)-visible spectrophotometer. A calibration curve was prepared at the range of 2-20 µg/ml and amount of folic acid in extract was estimated.[11]

Estimation of iron

A total of 100 mg of extract was transferred in 10 ml of water and transferred into a 100 ml volumetric flask, 5 ml of 2.058 mol/ml potassium thiocynate and 3 ml of 6 mol/ml nitric acid were added to develop the color. E volume was made to 100 cm^3 mark with de ionized water. Absorbance readings were measured for each including the standard solutions at 579 nm using UV-visible spectrophotometer.[12]

Estimation of vitamin C

Five grams of extract was homogenized with 25 ml of metaphosphoric acid-acetic acid solution, and it was quantitatively transferred into a 50 ml volumetric flask and shaken gently to homogenize solution. Later, it was diluted up to the mark by the meta phosphoric acid-acetic acid solution. The obtained solution is filtered and centrifuged at 4000 rpm for 15 min, 0.23 ml of 3% bromine water were added into 4 ml of centrifuged sample solution to oxidize the ascorbic acid to dehydroascorbic acid and after that 0.13 ml of 10 % thiourea to remove the excess of bromine. Then, 1 ml of 2, 4-dinitrophenylhydrazine solution was added to form osazone kept at 37°C temperature for 3 h in a thermostatic bath. After it was cooled in ice bath for 30 min and treated with 5 ml chilled 85 % H_2SO_4, with constant stirring. As a result, a colored solution's absorbance was taken at 521 nm. Concentration of ascorbic acid was determined using calibration of standard ascorbic acid (2-10 µg/ml).[13]

Acute toxicity studies and selection of dose

Jain and Singhai[14] reported the oral dose of 100 and 200 mg/kg of ethanol extract of beetroot for *in vivo* experiments determined as per organization for economic co-operation and development acute toxicity studies guidelines. Hence in the present work, same dose viz. 100 mg/kg and 200 mg/kg of extract was taken for studies.

Induction of experimental anemia

Anemia was induced by intraperitoneal injection of phenyl hydrazine (60 mg/kg, i.p., in divided doses daily, for 3 consecutive days.[15,16] Anemia was considered to be induced when red blood cell (RBC) level as well as hemoglobin concentration of the blood reduced by about 30%.

Study group design

After induction of anemia, rats were divided into groups, except Group I comprised of healthy rats. Grouping is as follows:

- Group I: Normal control
- Group II: No treatment
- Group II: Standard treatment (Ferritop-Z)
- Group III: Extract (100 mg/kg)
- Group IV: Extract (200 mg/kg).

Bio-analytical studies

The RBC number and hemoglobin concentration were determined every 3 days for 24 days.

Statistical analysis

Data are presented as mean ± standard error mean and analyzed by using one-way analysis of variance followed by Bonferroni's multiple comparison test (post-test); $P \leq 0.05$ was considered as statistically significant in all analyses.

RESULTS

Extraction and phytochemical screening

The yield of extract was 4.3 g. Extract was pale-brown in color. The results of preliminary phytochemical analysis

extract of *B. vulgaris* showed abundant presence of alkaloids, terpenoids, saponins, tannins, and polyphenols.

Phytoanalytical studies

Contents of phytoconstituents estimated are as follows:

- Folic acid: 2.21±0.54 µg/g of extract
- Ascorbic acid: 3.78±0.26 mg/g of extract
- Iron: 30.42±3.17 mg/g of extract.

Erythropoietic effect

Following the induction of anemia, the number of erythrocytes and the hemoglobin concentration decreased by 62.51% and 69.64%, respectively. Administration of standard hematinic preparation and extract (200 mg/kg) resulted in significant increase ($P < 0.001$; $P < 0.01$) in the number of RBCs as well as hemoglobin concentration when compared to the untreated phenylhydrazine-induced anemic rats (Figures 1 and 2).

DISCUSSION

Para substitution on phenyl rings is responsible for imparting nucleophillic character to phenyl hydrazine.[17] During the process of oxidation, it forms free radicals.[18] When enters into blood stream, it causes hemolysis, which arise due to oxidative alterations of blood cell proteins.[19] This process leads to premature aging of erythrocytes and predisposes to premature splenic sequestration. This leads to lack of circulating erythrocytes and hemoglobin.[20]

In the present work, anti-anemic activity of *B. vulgaris* was determined. Beetroot is rich source of folic acid, ascorbic acid, and iron. Folic acid/folate is a water soluble vitamin. It is found abundantly in green leafy vegetables, fruits, grains, and cereals.[21] A healthy person requires 200-400 µg folic acid per day, however, during pregnancy and lactation, its daily requirements become 500-600 µg.[22] Deficiency of folic acid in body may lead to pathogenesis of megaloblastic anemia and macrocytosis of erythrocytes.[23] Diet low in folic acid may increase risk of breast, pancreatic, and colon cancer.[24-26] Its low level in body may also predispose to coronary artery disease.[27] Ascorbic acid is an important component of human diet. It is an established fact that ascorbic acid is involved in release of iron from ferritin.[28] Reduced level of ascorbic acid and its decreased catabolism is also a condition observed during anemia.[29]

Diminution of levels of iron is one of the major hallmarks of anemia. According to a report of World Health Organization, about 35-75 % of pregnant women suffer from anemia.[30] This condition is observed when iron demand by body is more than its availability in body. This could be also due to insufficient iron intake, impairment in mechanism of iron absorption and transportation and/or chronic blood loss.[31]

In this work, reference hematinic preparation (Ferritop-Z) contains iron that forms an integral part of hemoglobin. Various vitamins from B vitamin family are responsible to a precursor in creation of cofactors for hematopoiesis. Extract effectively raised the level of hemoglobin

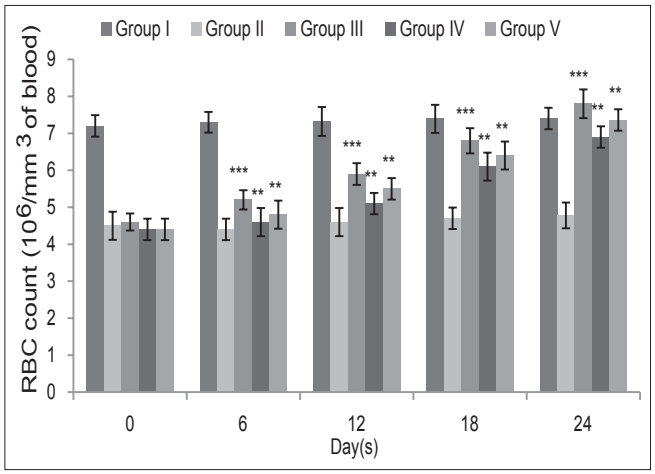

Figure 1: Effect of *Beta vulgaris* extract on red blood cell count in phenylhydrazine induced anemic rats. Data are presented as mean ± standard error mean and analyzed by one-way analysis of variance followed by Bonferroni's multiple comparison test (post-test); $P \leq 0.05$ was considered statistically significant in all analyses.

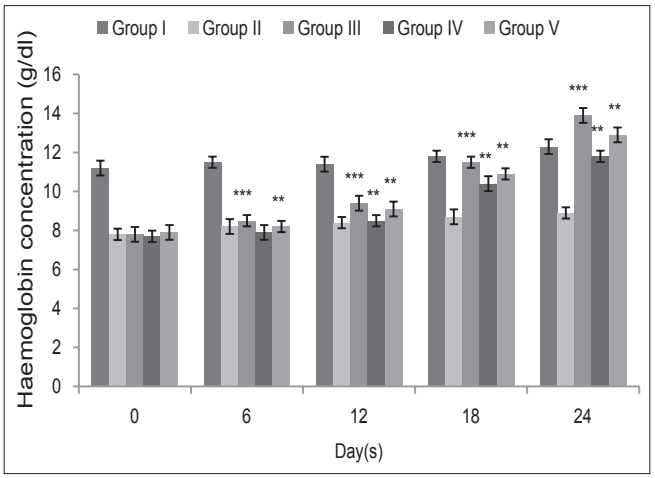

Figure 2: Effect of *Beta vulgaris* extract on hemoglobin concentration in phenyl hydrazine induced anemic rats. Data are presented as mean ± standard error mean and analyzed by one-way analysis of variance, followed by Bonferroni's multiple comparison test (post-test); $P \leq 0.05$ was considered as statistically significant in all analyses.

and erythrocyte count (at dose 200 mg/kg). Vitamin and minerals found in beetroot are most likely active ingredients responsible for its hematinic effects. Still, methodical studies are obligatory to derive its effects on humans.

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Source of Support: Authors deuly acknowledge Rewa Shiksha Samiti for kind support during studies, **Conflict of Interest:** None declared.