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The Ethanolic Extract of Beetroot (*Beta Vulgaris)* Ameliorates Some Red Cell Parameters In Phenylhydrazine-Induced Anaemic Rats

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Abstract: *Beetroot is a plant with high nutritional and medicinal value. This study investigated the anti-anaemic effect of beetroot on wistar rats. 25 male wistar rats were randomly assigned into 5 groups (n=5) thus: control, anaemic, beet$_{(only)}$, beet$_{(LD)}$ and beet$_{(HD)}$ groups. Except for control (0.2ml normal saline) and beet$_{(only)}$ groups, other groups were induced with anaemia using phenylhydrazine (60mg/kg) intraperitoneally on days 1-3. Beet$_{(only)}$, beet$_{(LD)}$ and beet$_{(HD)}$ groups received oral standard (200mg/kg), low (100mg/kg) and high (400mg/kg) doses of beetroot extract respectively daily for 14 days. Results show that RBC count, Hb concentration and PCV decreased by (38%, 31% and 20% respectively) in anaemic group compared with control. RBC, Hb concentration and PCV increased in beet$_{(LD)}$ (68%, 57% and 30% respectively) and beet$_{(HD)}$ (87%, 69%,and 35%respectively) groups compared with anaemic group. MCV increased (36%) in anaemic group compared with the control but decreased in beet$_{(LD)}$ (23%) and beet$_{(HD)}$ (24%) groups compared with anaemic. MCHC, decreased in the anaemic (8%) but increased slightly in the Beet$_{LD}$ (7%) and Beet$_{HD}$ (8%) groups compared with the anaemic group. All the red cell indices increased slightly in the beet$_{ONLY}$ group compared with control. Beetroot extract demonstrated anti-anaemic effect and is effective in defending the body against anaemia associated diseases.*

Keywords: *Anaemia, Beetroot, phenylhydrazine, red blood cell*

List of abbreviations: *red blood cell (RBC), haemoglobin (Hb) , packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), beet$_{(only)}$ (only beet root), beet$_{(LD)}$ (low dose of beetroot), beet$_{(HD)}$ (high dose of beetroot)*

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I. Introduction

Several plants are being used globally as foods and for medicinal purposes. Medicinal plants have recorded great potential in the management and treatment of various health problems. An example of such plants is the beetroot. Beetroot, (*Beta vulgaris)*, is a plant that belongs to the family, Chenopodiaceae [1]. Beetroot has been reported to possess anti-depressant [2], hepatoprotective [3, 4], anti-hypertensive [5], antioxidant [6], anti-hyperlipidaemic[7], radioprotective and immunostimulatory [8] effects. It also possesses anti-cancer, immunomodulatory, anti-inflammatory, anti-mutagenic, anti-microbial and anti-fungal activities and is used as expectorant and carminative [1]. Renal dysfunction and structural damage have been attenuated using beetroot [9]. Beetroot has been reported to enhance the proliferation of irradiated splenocytes and inhibit DNA damage in splenocytes induced by irradiation in mice [8]. The juice is consumed as a natural treatment for sexual weakness and to remove kidney and bladder stones [10]. Beetroot is also consumed in salad with other vegetables worldwide [11]. From the foregoing, it is clear that beetroot is a plant with high medicinal value. Hippocrates, the father of medicine, recommended the leaves of beetroot for faster wound healing [12].

These medicinal properties of beetroot are attributed to their phytochemical and mineral composition. Alkaloids, flavonoids, phenols, coumarins, triterpenes, tannins, fatty acids, saponins, anthocyanins, beta carotene, amino acids and vitamins A, C, E and K have been identified in beetroot along with minerals such as magnesium, copper, calcium, iron, potassium, manganese and folic acid [1, 13].

Beetroot has also demonstrated anti-anaemic effect. In a study conducted by some researchers [8] on mice, beetroot (400mg/kg) was shown to inhibit irradiation-induced apoptosis and stimulate proliferation of haematopoietic progenitor cells via inducing the secretion of cytokines associated with haematopoietic progenitor cell stimulation. Their study showed that beetroot significantly increased the total number of bone marrow cells, red blood cell (RBC) count, haemoglobin (Hb) concentration and packed cell volume (PCV) compared to the irradiated group thus demonstrating the anti-anaemic effect of beetroot. In another study conducted by Jaiswal and others [14] where phenylhydrazine was used to induce anaemia in rats, beetroot (200mg/kg) demonstrated anti-anaemic effect by significantly increasing RBC count and Hb concentration in

anaemic rats treated with beetroot compared with the phenylhydrazine-induced anaemic rats. There is need for further studies on anti-anaemic effect of beetroot using low and high doses in order to firmly support the few available studies. The present study was however conducted to investigate the effect of low, and high doses of beetroot ethanolic extract on red blood cell parameters of phenylhydrazine-induced anaemic wistar rats.

MATERIALS AND METHODS

Extract Preparation and Purchase of Drug

Beetroots (*Beta vulgaris)* were purchased from Marian market, Calabar, Nigeria. They were washed with cold running water and gently rubbed with a towel to remove dirt. They were then peeled, cut into smaller pieces and pulverised into fine particles using an electric blender. The particles were macerated in 2800mL of ethanol for 24 hours, filtered and the filtrate was concentrated to dryness using an oven (AstellHearson, England) at 40°C - 50°C and stored in a refrigerator until it was used. Phenylhydrazinewas purchased from Bez Pharmacy, Etta Agbor, Calabar, Nigeria.

Ethical approval: This research related to animal use, had been complied with all the relevant national regulations and institutional policies for the care and use of animals

Experimental Animals

Twenty-five male wistar rats (200-250g) were utilized for the study. The animals were purchased from Department of Agriculture, University of Calabar. They were handled according to Helsinki's [15] laid down principles, housed in properly ventilated metallic cages in the animal house of Physiology Department, University of Calabar. The rats were given standard rat feed and water *ad libitum* and exposed to 12/12 hours light/dark cycle at 28.0 ± 2°C. They were allowed for fourteen days to acclimatise before commencement of treatment. The study was approved by the Ethics Committee of Faculty of Basic Medical Sciences, University of Calabar.

Experimental Design

The 25 male wistar rats were randomly assigned into 5 groups (n=5) thus: control, anaemic, $beet_{(only)}$, $beet_{(LD)}$ (low dose) and $beet_{(HD)}$ (high dose) groups. All groups received rat feed and water. In addition, the control group received 0.2mL normal saline orally. Anemia was induced using intraperitoneal injection of phenylhydrazine(60mg/kg body weight) on days 1-3 [14]. The $beet_{(only)}$ group received only mean dose of beetroot extract (200mg/kg body weight) with no induction of anaemia [14]. In the $Beet_{(LD)}$ group, anemia was induced in the same way as in the anaemic group and the rats received low dose of beetroot ethanolic extract (100mg/kg body weight). Rats in the $beet_{(HD)}$ group were administered phenylhydrazine and received high dose of the beetroot ethanolic extract (400mg/kg body weight). The doses used in this study are as previously used [8, 16]. Extract was administered orally for 14 days.

Collection of Blood Samples

After the fourteen-day administration, the rats were sacrificed under 3.8 % chloroform anaesthesia. Blood samples were collected via cardiac puncture using 5mL syringes with 21G needles into ethylenediaminetetracetate (EDTA) coated vials and gently agitated to ensure uniform spread of EDTA. The samples were thereafter used for measurement of blood parameters.

Measurement of Haematological Parameters

Automated cell counter (Coulter Electronics, Luton, Bedfordshine, UK) was used to measure haematological parameters. Parameters measured were: red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC)

Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Data were analyzed using Statistical Package for Social Science (SPSS) (version 17). Statistics used was one way analysis of variance (ANOVA) followed by post hoc multiple comparison. $p<0.05$ was considered statistically significant.

II. Results

Red Blood Cell Indices

Table 1 shows RBC count ($x10^6$ cell/µL), Hb concentration (g/dL), PCV (%), MCV (fL), MCH (pg) and MCHC (g/dL) for control, anaemic, $beet_{(only)}$, $beet_{(LD)}$ and $beet_{(HD)}$ groups. RBC count decreased (38%) in anaemic group compared with the control; but increased slightly (9%) in the $beet_{ONLY}$ group compared with

control. When the RBC of the $beet_{(LD)}$ and $beet_{HD}$ groups were compared with the anaemic group, there was an increase (68% and 87% respectively). Hb and PCV concentrations were also decreased (31% and 20% respectively) in the anaemic group when compared with the control. The Hb of $beet_{(LD)}$ and $beet_{(HD)}$ groups increased (57% and 69% respectively) when compared with anaemic group. The $beet_{LD}$ and $beet_{HD}$ groups also presented a 30% and 35% increase respectively in PCV compared with anaemic group. The Hb and PCV of the $beet_{only}$ group increased slightly (8%) when compared with the control. MCV was increased in anaemic (36%) and $beet_{(only)}$ (1%) groups when compared with the control. The MCV of $beet_{(LD)}$ and $beet_{(HD)}$ groups reduced (23% and 24% respectively) when compared with the anaemic group. There was an increase in MCH in $beet_{only}$ group (10%) when compared with the control but MCH decreased in the anaemic group (11%) when compared with control. The MCH of the $beet_{LD}$ and $beeet_{HD}$ groups were increased (29% and 45% respectively) when compared with the anaemic group. There was a slight decrease in MCHC in the anaemic group (8%) but a slight increase in MCHC in the beetonly (2%) group when compared with the control. In the beetLD and beetHD groups, there was a slight increase in MCHC (7% and 8% respectively) when the two groups were compared with the anaemic group.

Table no1: Comparison of red blood cell indices between the different groups

Parameter	Control	Anaemic	$beet_{(only)}$	$beet_{(LD)}$	$beet_{(HD)}$
RBC ($x10^6$ cell/µL)	8..20±0.12	$5.05±0.19^{38\%C}$	$9.03±0.36^{9\%}$	$8.50±0.18^{68\%}$	$9.45±0.17^{87\%}$
Hb (g/dL)	14.89±0.12	$10.26±0.07^{31\%}$	$16.23±0.61^{8\%}$	$16.09±0.35^{57\%}$	$17.32±0.26^{69\%}$
PCV (%)	48.04±0.44	$38.62±0.76^{20\%}$	$52.31±1.99^{8\%}$	$50.31±1.14^{30\%}$	$52.30±0.96^{35\%}$
MCV (fL)	50.08±0.10	$68.56±2.49^{36\%}$	$50.81±0.70^{1\%}$	$52.56±1.38^{-23\%}$	$51.54±0.96^{-24\%}$
MCH (pg)	14.90±0.12	$13.33±1.39^{-11\%}$	$16.61±0.50^{10\%}$	$17.23±0.26^{29\%}$	$19.28±0.14^{45\%}$
MCHC (g/dL)	31.56±0.30	$30.45±0.87^{-8\%}$	$32.32±0.41^{2\%}$	$32.53 ± 0.30^{7\%}$	$32.90±0.41^{8\%}$

Values are expressed as mean ± SEM, n = 5.
Percentage differences between anemic and control, beet alone and control, beetLD and anaemic; beetHD and anemic are represented by the figures seen in superscript against anaemic, beet alone, beetLD and beetHD values respectively.

III. Discussion

Beetroot is a plant with high medicinal value. This study investigated the effect of low, high doses of ethanolic extract of beetroot on red cell parameters of phenylhydrazine-induced anaemic wistar rats.

RBC count was 38% decreased in anaemic and $beet_{(LD)}$ groups compared with control. It was not very different in $beet_{(only)}$ (9%), compared with the control. However, when $beet_{(LD)}$ and $beet_{(HD)}$ groups were compared with anaemic group there was an increase in RBC (68% and 87% respectively) (**Table 1).** These results indicate that phenylhydrazine caused destruction of red blood cells (RBCs) (Macmillan et al [17]). Also, beetroot at 100mg and 400mg/kg were able to prevent the effect of phenylhydrazine as there was an increase (68% and 87% respectively) in RBCs in $beet_{LD}$ and $beet_{(HD)}$ groups compared with anaemic group. This is in line with the results of Jaiswal et al. [14], who induced anemia by administering divided doses of 60mg/kg phenylhydrazine for three days consecutively and ameliorating with two doses of ethanolic beetroot extract [100mg/kg and 200mg/kg]. The above study showed that beetroot significantly increased RBCs count in anaemic rats compared with untreated phenylhydrazine-induced anaemic rats. Inducement of anaemia was confirmed as the anaemic group presented a 31% decrease in Hb concentration compared with control which is in tandem with Jaiswalet al. [14]. This decreased Hb concentration, induced by phenylhydrazine, is probably due to the decreased RBCs count or impairment of heme biosynthesis. However, beetroot at the administered doses prevented this possible negative effect on Hb as Hb concentration was significantly increased in $beet_{(LD)}$ and $beet_{(HD}$ (68% and 87% respectively) groups compared with anaemic group. This result corroborates with that of Jaiswalet al. [14] who also reported that beetroot administered to anaemic rats significantly increased Hb concentration and PCV compared to untreated phenylhydrazine-induced anaemic rats. It would however appear that the ability of beet root to stimulate erythropoiesis and restore red cell parameters to normalcy is more potent in the anaemic state than in normalcy. There may therefore be stimulant present during anaemic states that accentuates the anti-anaemic actions of beetroot. The result is also consistent with Cho et al. [8] who reported that Hb concentration and PCV significantly increased in anaemic mice treated with beetroot (400mg/kg) compared with irradiation-induced anaemic mice. Indhumathi and kannikaparameswari [16] also reported significant increase in Hb concentration and PCV in a dose dependent fashion in normal rats treated with methanolic extract beetroot (100, 200 and 400mg/kg) administered for 16days .This indicates the tendency of beetroot to prevent iron deficiency anaemia. Beetroot contains folic acid and folic acid is necessary for the absorption of iron from the gastrointestinal tract for synthesis of Hb. High dose of beetroot not only prevented the negative effect of phenylhydrazine on Hb but also probably improved the biosynthesis of Hb as Hb concentration was significantly increased in $beet_{(HD)}$ group compared with control and $beet_{(only)}$ groups. PCV was decreased in

anaemic group compared with control and increased in $beet_{(LD)}$ and $beet_{(HD)}$ groups compared with anaemic group (**Table 1)**. This agrees with the report of Jaiswal *et al.* [14]. The decrease in PCV in anaemic group is probably due to the decreased RBCs count associated with this group. These results again show the anti-anaemic effect of beetroot. The effect is greatest with high dose of beetroot as seen in this study where PCV was increased to a greater extent in $beet_{(HD)}$ group than in the $beet_{LD}$ group when compared with the anaemic group.

IV. Conclusion

Beetroot at the administered doses exhibited anti-anaemic effect which was more potent in anaemia. It is therefore effective in the treatment of anaemia

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