NISEB Journal Vol. 17, No. 3. September, 2017 1595-6938/2017
Printed in Nigeria (2017) Society for Experimental Biology of Nigeria
http://www.nisebjournal.org

Toxicity Study of Beetroot (*Beta vulgaris*) Extract in Normal Sprague Dawley Rats

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Abstract
The safety of beetroot as a health promoting and disease preventing functional food was evaluated. Freeze dried beetroot was extracted with distilled water. Different doses of 10-1000 mg/kg and 1600-5000 mg/kg body weight were administered orally as a single dose (acute toxicity). Doses of 200-3000 mg/kg body weight/day for 28 days were administered orally (subchronic toxicity) as against distilled water in the control group. The LD_{50} was greater than 5000 mg/kg body weight. Animals were euthanized and examined for biochemical and haematological changes. Feed intake was normal, but the body and organ weights were significantly ($p<0.05$) increased. There was a significant ($p<0.5$) decrease in serum blood glucose levels at 500 and 1000 mg/kg body weight of extract. Platelets and lymphocytes were significantly ($p<0.05$) decreased at lower doses of extract, while WBC, monocyte and granulocyte increase were dose dependent. The level of MCHC was significantly ($p<0.05$) increased in the test groups when compared to control. The extract consumption is safe, and possesses hypoglycaemic potentials.

Keywords: Beetroot, Toxicity, Safety, Dose, Extract

Introduction
Beetroot *(Beta vulgaris)* also known as the common beet, garden beet, red beet, or table beet is a root vegetable in the *Chenopodiaceae* family. Other vegetables in this family include spinach, chard, quinoa, and sugar beets [1]. It is noted that not all medicinal plants are safe for consumption in the crude form. Some level of toxicity may arise as a result of potential toxic compounds they contain and the application of pesticide during cultivation [2,3]. Beetroot is grown widely in Germany and France and in lesser amounts in other European countries, Africa, Asia and South America. It is now a popular salad vegetable. Beetroot is a true biennial that produces thickened root and a rosette of leaves during the first year followed by flowers and seeds during the second year. They are mainly grown for their swollen roots but the leaves can also be eaten as spinach. The flowers are very small with a diameter of 3 to 5 mm and are produced in dense spikes. They are green or tinged reddish, with five petals. The fruit is a cluster of hard nut lets [4]. Besides other active chemicals, beetroots contain a unique class of water-soluble, non-phenolic antioxidants, the betalains, red betacyanins and yellow betaxanthines [5]. Beetroots have been shown to contain a variety of minerals : calcium, iron, magnesium, potassium, selenium, zinc) and vitamins (vitamin C, thiamin, vitamin B6, vitamin A, beta carotene, vitamin E, and vitamin K [6].
Studies have shown that many African countries still rely on traditional medicine to meet different health needs [7]. Extracts of plants are used for the treatment of various diseases which forms the basis for all traditional systems of medicine [8]. Recent reports indicate that *Beta vulgaris* extracts (root) possess antihypertensive, antioxidant [9], anti-inflammatory, and hepatoprotective activities [10, 11, 12, 13]. Previously, red beetroot extract has been demonstrated to be an effective multiorgan tumor suppressing agent in laboratory animals [12, 14, 15].
One major and overriding criterion in the selection of herbal medicines for use in health services is safety. Plants extracts should not only be efficacious but safe for consumption [16]. To ensure the safety of these products given their rapidly increasing use, there is need to assess the risk associated with herbal medicine and products derived from them [17]. The toxic effects produced by the administration of drugs as derivatives of these plants are much more a serious problem than the disease itself [18]. Although *Beta vulgaris* is used worldwide in traditional medicine, toxicological data on the plant are scarce. This study was designed to assess the toxicity of the root aqueous extract in Sprague Dawley rats, with the purpose of providing information on the safe use of this plant.

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Materials and Methods

Plant material

Beetroot was obtained from a local vendor at the vegetable market on Airport road, Benin City, Nigeria. The plant was identified by a Taxonomist at the Department of Plant Biology and Biotechnology at the University of Benin, Benin City, Nigeria. A voucher number UBH_B 374 was obtained and deposited in the Herbarium. The beetroots were washed thoroughly so as to remove any of the mud or impurities from the surfaces, peeled using a kitchen knife and chopped finely into small bits of about 2cm each. The chopped beetroot was blended using an electric Moulinex Blender LM 2411, Wanette, Oklahoma, USA. Distilled water (200-300 ml) was added to make it into a smooth consistent paste or juice, until no solid beetroot was visible. The juice was extracted using a muslin cloth and stored in a plastic container, rapped with black cellophane so as to prevent fermentation and auto-oxidation. The juice obtained was freeze dried [Armfield vacuum freeze dryer Model FT 33, Ringwood, England] and ready for use. The freeze dried sample of 10% beetroot juice was evaluated to contain 9808.0 mg GAE/100 ml polyphenols and 8334.0 mg QE/100 ml flavonoids [19].

Animals

Healthy male and female Sprague Dawley rats of average weight [135-185.0 g] were obtained from the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. They were maintained according to the guidelines of Committee for the Purpose of Control and Supervision of experiments on animals [20]. The animals were kept in clean plastic cages that were floored with animal beddings. The animals had a 12 h light/dark cycle and the beddings changed daily. The animals were grouped according to sex and weight. The rats were fed with pelletized poultry finisher's mash feed (Top Feeds, Ibadan, Nigeria). Approval for this study was obtained after review of the protocol by the Ethical Committee, College of Medical Sciences, University of Benin, Benin City, Nigeria, with number CMS/REC/2016/002**.**

Acute oral toxicity

The evaluation of acute oral toxicity of *Beta vulgaris* was carried out using Lorke's method [21]. Fifteen (15) rats were used for this study and involved two phases; in phase I, there were three treatment groups with three rats per group as against control group that had distilled water. The extract was administered as follows: group I (normal rats that received distilled water), groups II, III and IV were orally administered 10 mg, 100 mg and 1000 mg/kg body weight of aqueous extract respectively in a single dose using gastric gavage. In phase II, there were three treatment groups of one animal each (group V, VI and VII), and were orally administered 1600 mg, 2900 mg and 5000 mg/kg body weight of extract respectively in a single dose using gastric gavage. The animals were observed for mortality, signs of gross toxicity and behavioral changes one hour post dosing and at least once daily for 14 days. Body weights and feed intake were recorded before dosing and after the observation period.

Subchronic toxicity

Six groups of five (5) animals each distributed according to weight (average body weight 135.0-185.0 g) and sex. The subchronic toxicity study was for twenty-eight (28) days with daily administration of aqueous extract of beetroot. The test groups received orally aqueous extract dissolved in distilled water at 200, 500, 1000, 2000 and 3000 mg/kg body weight/day/rat. The normal control animals had distilled water. The animals were observed for signs of toxicity and mortality throughout the experimental period. At the 28^{th} day the animals were fasted for 12 h and euthanized by decapitation. Blood was collected for biochemical and haematological evaluation, while the kidney, liver and pancreas were dissected, freed of adherent tissues and weighed.

Blood analysis

Fasting blood glucose was determined in all the groups using the a glucometer (ACCU-Check, Roche, Germany) on day 0, 7, 14, 21 and 28 using caudal vein blood samples. Serum glucose was determined using the Randox kit (glucose oxidase). White blood cell (WBC), total and differentials, red blood cell (RBC), platelet counts, haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were determined using an automatic blood analyser (URIT-3010 Automated Hematology Analyzer, Gullin, Guangxi, China).

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). The difference between the groups was tested using ANOVA. Duncan's multiple range test was used to test for significant difference among the means ($p < 0.05$).

Results

Acute oral toxicity

Though toxicity was monitored by mortality, other means included changes in the skin, salivation, tremors, refusal of feed, bleeding from the orifices, diarrhea and paralysis. In phases I and II of this study, none of the toxicity indices mentioned above were observed. After 14 days of observation in the phases of the acute toxicity study, there was no mortality or death recorded at the 5000 mg/kg/body weight of extract (Table 1). Therefore,

the LD_{50} value of *Beta vulgaris* extract administered orally is higher than 5000 mg/kg body weight. The method described by [21] was used to determine acute toxicity of the extract in Sprague Dawley rats.

Table 1: Effect of aqueous extract of beetroot on weight changes in Sprague Dawley rats.

PHASE I

Groups	Dosage (mg/kg)	Day 0	Day 14	% Weight Gain
Group I (Control)	Distilled water	151.73±0.87[a]	162.70±1.27[a]	7.22±0.25[b]
Group II	10	162.67±4.55[a]	172.57±6.02[a]	6.05±0.73[ab]
Group III	100	163.03±5.76[a]	170.03±6.29[a]	4.30±1.25[a]
Group IV	1000	160.80±5.23[a]	166.87±4.29[a]	3.82±0.72[a]
PHASE II				
Group V	1600	167.6	175.3	7.7
Group VI	2900	163.6	172.9	9.3
Group VII	5000	157.3	158.6	1.3

*Values in the same column that have different alphabets are statistically significant (P<0.05)

Subchronic Toxicity

Clinical observations

Daily oral administration of aqueous extract of *Beta vulgaris* extract at tested doses (200, 500 1000, 2000 and 3000 mg/kg body weight) for 28 days, did not induce any obvious symptoms of toxicity or mortality in the rats.

Biochemical assessment

The blood glucose levels in the groups administered 500 and 1000 mg/kg of extract were significantly ($p < 0.05$) decreased when compared to their controls (Table 2). The average body weight of the animals administered the extracts were significantly ($p < 0.05$) increased when compared to control group (Figure 1). The feed consumed by the animals at 200, 500 and 3000 mg/kg of aqueous extract of beetroot were significantly ($p<0.05$) increased when compared to control (Figure 2). At 500 mg/kg of extract the relative organ weights of kidney, liver and pancreas were significantly ($p<0.05$) increased when compared to the control (Table 3).

Table 2: Effect of repeated daily oral administration of aqueous extract of beetroot for 28 days at different concentrations on blood glucose of Sprague Dawley rats

	Control	**Group 1 (200 mg)**	**Group 2 (500 mg)**	**Group 3 (1000 mg)**	**Group 4 (2000 mg)**	**Group 5 (3000 mg)**	**p**
			Concentration of blood glucose (mg/dl)				
Day 0	38.20±5.78[a]	65.00±3.21[b]	68.80±3.07[b]	69.00±7.48[b]	83.40±5.57[b]	72.40±2.50[b]	0.000
Day 7	49.80±2.48[a]	39.00±6.17[a]	41.60±4.01[a]	46.20±3.04[a]	41.40±1.29[a]	46.75±7.33[a]	0.490
Day 14	56.20±2.31[a]	43.80±1.66[a]	46.60±4.18[ab]	42.50±3.20[a]	47.80±2.31[a]	29.00±2.35[b]	0.000
Day 21	66.00±3.37[a]	46.00±2.45[b]	40.80±2.35[b]	43.00±3.29[b]	42.25±2.10[b]	38.50±1.26[b]	0.000
Day 28	65.20±2.01[a]	54.40±2.20[b]	35.75±1.93[c]	42.50±1.71[c]	46.00±2.80[bc]	45.00±1.08[bc]	0.000

Values are expressed as mean ± SEM of 5 rats .Values carrying different notations are statistically different at *$p < 0.05$. Data was obtained at 0 time, 7, 14, 21 and 28 days.

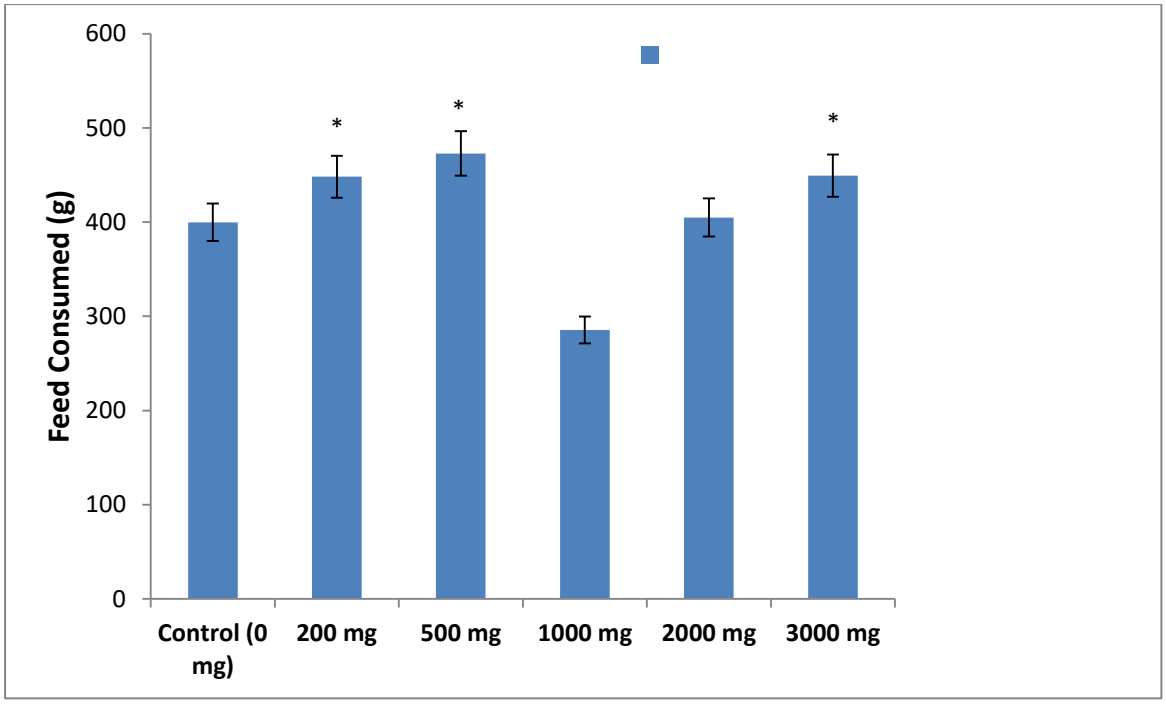

Figure 1: Effect of oral administration of different doses of the extract on feed intake in Sprague Dawley rats. Result was expressed as mean ± SEM. *p < 0.05 is significant compared to control.

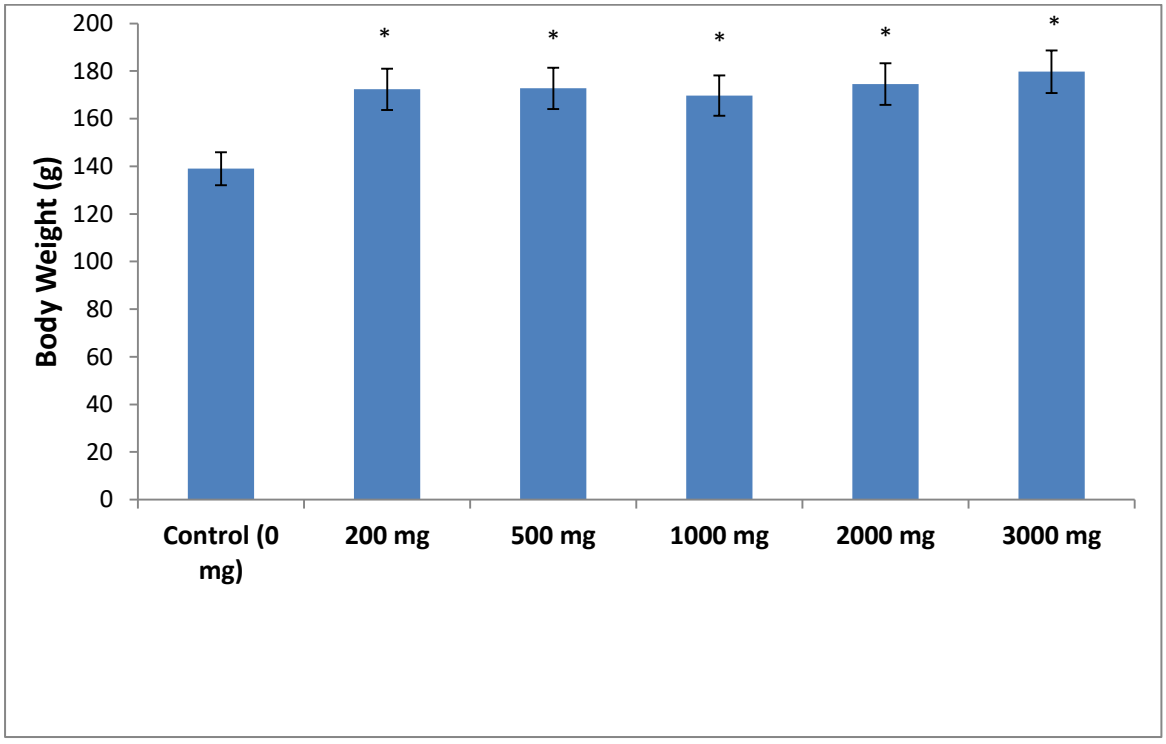

Figure 2: Effect of oral administration of different doses of the extract on body weight in Sprague Dawley rats. Body weights were expressed as mean ± SEM. *p < 0.05 is significant compared to control.

Table 3: Effect of oral administration of different doses of the extract on relative organ weights of rats.

Weight of organ (g)	Control (0 mg)	Group 1 200 mg	Group 2 500 mg	Group 3 1000 mg	Group 4 2000 mg	Group 5 3000 mg
Kidney	0.30 ± 0.01^{a}	0.35 ± 0.02^{ab}	0.40 ± 0.02^{b}	0.34 ± 0.03^{ab}	0.31 ± 0.02^{a}	0.28 ± 0.02^{a}
Liver	2.92 ± 0.09^{a}	2.74 ± 0.07^{ab}	3.00 ± 0.11^{b}	2.57 ± 0.12^{a}	2.70 ± 0.09^{ab}	2.61 ± 0.07^{a}
Pancreas	0.34 ± 0.02^{a}	0.34 ± 0.05^{a}	0.53 ± 0.05^{b}	0.44 ± 0.07^{ab}	0.33 ± 0.03^{a}	0.33 ± 0.01^{a}

Data are expressed as mean ± SEM of 5 rats. Values carrying different notations are statistically different at *p < 0.05. Beetroot extract was administered daily for 28 days by gastric gavage.

Haematological Analysis

The level of MCHC was significantly increased ($p<0.05$) in all the tested doses of the extract when compared to normal rats (Table 4). The levels of RBC, PCV, Hgb, MCV and MCH were not statistically ($p>0.05$) significant (Table 4). Nevertheless, the platelet counts at 200 and 500 mg/kg body weight of beetroot extract were significantly ($p<0.05$) reduced, while the WBC was significantly ($p<0.05$) increased at 500 mg of extract when compared to 200, 1000, 3000 mg/kg (Table 5). The level of monocytes were significantly ($p<0.05$) increased at 200 mg of extract when compared to control (Table 5). However, granulocytes were significantly ($p<0.05$) increased at the tested doses of 200, 500 and 1000 mg/kg of extract (Table 5).

Table 4: Effect of oral administration of different doses of extract on Red Cell Indices in Sprague Dawley rats

GROUPS	RBC ($\times 10^3$/µl)	HGB (g/dl)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control (0 mg)	5.65 ± 1.14^{a}	13.40 ± 1.88^{a}	40.20 ± 5.62^{a}	79.90 ± 7.83^{a}	26.58 ± 2.58^{a}	33.08 ± 0.05^{a}
200 mg	5.28 ± 0.72^{a}	12.78 ± 1.53^{a}	38.32 ± 4.56^{a}	73.90 ± 1.60^{a}	24.64 ± 0.52^{a}	33.28 ± 0.07^{b}
500 mg	4.74 ± 0.65^{a}	11.48 ± 1.44^{a}	34.42 ± 4.28^{a}	72.92 ± 1.70^{a}	24.32 ± 0.57^{a}	33.30 ± 0.05^{b}
1000 mg	5.33 ± 0.55^{a}	13.83 ± 1.06^{a}	41.45 ± 3.17^{a}	78.68 ± 4.71^{a}	26.35 ± 1.52^{a}	33.35 ± 0.03^{b}
2000 mg	5.44 ± 0.54^{a}	14.33 ± 1.60^{a}	42.95 ± 4.80^{a}	78.68 ± 1.88^{a}	26.30 ± 0.76^{a}	33.28 ± 0.06^{b}
3000 mg	5.56 ± 0.76^{a}	14.20 ± 1.92^{a}	42.63 ± 5.74^{a}	76.75 ± 2.88^{a}	25.58 ± 0.99^{a}	33.30 ± 0.41^{b}

Concentrations of RBC, Hgb, PCV, MCV, MCH, and MCHC were expressed as mean ± SEM of 5 rats. Values with different superscripts among the groups are significant ($P > 0.05$). Abbreviations: MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular haemoglobin concentration; Hgb, haemoglobin; PCV, pack cell volume; RBC, red blood cell.

Table 5: Effect of oral administration of different doses of the extract on white blood cells, platelets and differentials in Sprague Dawley rats

GROUPS	PLT (10^3µL)	WBC ($\times 10^6$/µl)	LYMH (%)	MON (%)	GRAN (%)
Control (0 mg)	489.50 ± 59.17^{b}	3.15 ± 0.89^{a}	88.03 ± 4.10^{b}	4.37 ± 0.64^{ab}	4.83 ± 0.90^{a}
200 mg	244.80 ± 49.92^{a}	4.20 ± 0.47^{ab}	66.50 ± 2.20^{a}	9.18 ± 1.02^{c}	24.30 ± 2.07^{b}
500 mg	275.00 ± 21.82^{a}	8.17 ± 1.25^{c}	77.46 ± 3.98^{ab}	6.04 ± 1.37^{abc}	18.08 ± 3.69^{b}
1000mg	359.50 ± 58.66^{ab}	2.75 ± 0.43^{a}	73.05 ± 8.62^{ab}	8.08 ± 1.72^{bc}	23.67 ± 7.68^{b}
2000 mg	350.75 ± 109.44^{ab}	6.13 ± 1.52^{bc}	86.08 ± 6.24^{b}	2.17 ± 0.34^{a}	5.67 ± 1.58^{a}
3000 mg	345.00 ± 58.97^{ab}	3.45 ± 0.45^{ab}	86.80 ± 3.13^{b}	4.80 ± 0.44^{ab}	13.33 ± 1.46^{ab}

Values in the same column with different superscripts are significant at $p < 0.05$.

Concentrations of WBC, LYMH, MON, GRAN and PLT were expressed as mean ± SEM of 5 rats. Abbreviations: PLT, platelets; WBC, white blood cell; LYMH, lymphocyte; MON, monocyte; GRAN, granulocyte.

Discussion

To determine safety of drugs and plant products for human use, toxicological evaluations are carried out in various experimental animals. The aim is to predict toxicity and provide guide lines for selecting a "safe" dose in humans [22]. Extracts of plants must be safe and effective. This study presents a comprehensive evaluation of aqueous extract of beetroot on acute and subchronic toxicity studies in normal Sprague Dawley rats. To determine the LD_{50} using the method of Lorke [21], a minimum dose of 10 mg and maximum dose of 5000 mg/kg body weight were administered in a single administration. In all the doses, the rats treated with the extract did not show any change in food consumption, behavioural patterns, body temperature, and respiratory rate. Signs such as salivation, diarrhoea and urination were not observed. There were comparable body weight gains among the rats treated with the extract when compared to those of control. All the treated rats survived until the 14^{th} day of observation. According to the acute toxicity grading standards [23], when LD_{50} is greater than 5000 mg/kg body weight, the extract is considered practically non-toxic. The findings from this study suggested that the LD_{50} was greater than 5000 mg/kg body weight. The absence of death among the rats in all the doses administered seemed to support this claim; therefore the single dose administration of the aqueous extract of *Beta vulgaris* can be said to have high safety profile when given orally.

The administration of beetroot extract for 28 days did not produce clinical signs of toxicity or mortality in the animals. Loss of appetite is often associated with weight loss which is due to the disturbance in carbohydrate, protein or fat metabolism [24]. Administration of high doses of crude plant extracts may be metabolised to toxic end products which could interfere with gastric function and decrease food conversion efficiency [25]. In this study the diet and water administered were well accepted by the animals treated with beetroot extract implying that it did not possibly alter carbohydrate, protein or fat metabolism in these experimental animals. A previous study [26], showed that *Euphorbia hirta L*. extract administered to rats did not adversely interfere with their nutritional benefits, weight gain and stability of appetite, which are expected for animals that are continually supplied with food and water *ad libitum*. There was a significant ($p < 0.05$) increase in feed consumption in the group of animals administered 200, 500 and 3000 mg/kg body weight of extract when compared to control, and this may be as a result of the tested doses (extract) not interfering with the feed consumption and utilisation. However, in the group of animals administered 1000 and 2000 mg/kg body weight of extract there was a significant ($p < 0.05$) reduction in feed consumption, which may be associated with the physiological state of the animals as this observation was dose dependent. There was a significant ($p < 0.05$) decrease in blood glucose in the animals administered 500 and 1000 mg/kg of extract, though the other tested doses showed a down regulation when compared to control. The reduction could be due to the presence of active ingredients in the extract which potentiated the hypoglycaemic effect observed.

Generally, when there is exposure to a toxic substance, a significant reduction in body weight by more than 10% from the initial body weight is a simple and sensitive index of toxicity [27]. Body weight changes is a sensitive indication of the general health status of animals [28]. Moreover, changes in body weight have been used as an indicator of adverse effects of drugs and chemicals [29]. The investigated doses of beetroot extract showed that there was a significant ($p < 0.05$) increase in the body weight of animals over a 28 day period when compared to control. The extract administered to the rats may have enhanced the absorption and utilisation of nutrients present in the animal feed. It is also possible that the active ingredients stimulated the appetite, and increased the utilisation of the feed consumed, which was observed by the weight gained in the animals. Hence prolong use of the extract could be safe and may not have any deleterious effect on the body weight of consumers of beetroot.

Organ weight is an important index of physiological and pathological status in animals, the heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reactions caused by toxicant [30]. The liver being a key organ in xenobiotics metabolism and detoxification is susceptible to damage induced by a variety of chemicals [30]. In this study the observed significant ($p<0.05$) increase in liver, kidney and pancreas at 500 mg/kg body weight of extract may be due to the active ingredients acting independently at this dose. It is suggested that the high rate of metabolism at 500 mg of extract could be responsible for the increased weight observed in these organs. However, if there was adverse reaction at the tested dose (500 mg), the feed consumption and body weight of the animals would have been significantly affected.

The analysis of blood parameters is relevant to risk evaluation, and changes in haematological system have a high predictive value for human toxicity when the data are translated from animal studies [31]. The assessment of haematological parameters can be used to reveal the deleterious effect of foreign compounds including plant extract on the blood constituents of animals [30]. The analysis of red blood cell indices in this study showed no significant ($p > 0.05$) changes following repeated administration of beetroot extract. This indicates that there were no lysis or inhibition of blood cell synthesis by any active ingredient present in the extract. The inability of the extract to impair erythropoiesis may be responsible for anaemia not developing even after prolonged use of the extract. Several workers have demonstrated that chronic treatment with plant extracts like *S. Lycopersicum, M. perennis, M. Annua, E. Laterifola, E.hyssopifola and Cassia italic* can cause destruction of haematological parameters leading to anaemia in mammals [32, 33, 34], however it had been reported [35] that a long term treatment of rats with *Teucrium polium* extract did not induce any toxic effect on red blood cells. It was then

suggested not to induce anaemia, which is in agreement with our study. Normally MCHC is associated with individual RBC, hence could stimulate the incorporation of haemoglobin into RBCs which ultimately bring about increase in oxygen exchange. The significant ($p<0.05$) increase in MCHC after 28 days of administration of extract may be due to the increased production of haematopoietic regulatory elements such as colony-stimulating factors. Since the oxygen-carrying capacity of the red blood cells may not have been compromised, the plant may be safe as used in traditional medicine.

White blood cells (WBC) or leukocytes are the cells in the immune system which play a role in body defence from foreign materials and infectious diseases. Leukocytes are produced in the haematopoietic stem cells also known as bone marrow. An increase in the number of white cells (leukocytosis) in the blood is a sign of infection or response to toxic environment [36]. The administration of plant extract can affect the principal function of phagocytes, which is to defend against invading microorganisms by ingesting and destroying them, hence contributing to cellular inflammatory processes [37]. The observed significant ($p<0.05$) increase of WBC at 500 and 2000 mg/kg body weight when compared to control may be due to the normal responses of rat to foreign bodies as it was not dose dependent. Though it has been reported that chronic treatment with some plant extracts caused a non-significant ($p>0.05$) increase in WBC as a normal response to the extract [38, 39]. Thrombocytosis (elevation of platelets) could result in spontaneous intravascular clotting and thromboembolism that may lead to complications of cardiovascular disease [40, 41]. When platelets are significantly decreased (thrombocytopaenia), there is tendency to bleed and have anticoagulant property [42]. In this study, the platelets were significantly ($p<0.05$) decreased at 200 and 500 mg/kg body weight of extract when compared to control. It is possible that some of the ingredients in the extract interacted differently with the blood cell line, as there were no variations in the other haematological parameters evaluated at the doses.

Conclusion

The administration of graded doses of beetroot extract to the animals did not result in mortality, neither was there any behavioural changes in the animals. The plant also has potential hypoglycaemic properties. All these observations may suggest that the plant is safe for medicinal use.

Acknowledgement

The authors are grateful to Tetfund for providing a grant that enabled the research.

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