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Assessment of risk of exposure from the consumption of *Glossocalyxbrevipes (Benth)* aqueous extract on the kidney of male wistar rats

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ABSTRACT

This study was designed to investigate the effect of Glossocalyxbrevipes (B) leaf and stem-bark extract on the kidney function of male Wistar albino rats. This investigation was based on the evaluation of risk of exposure using kidney function parameters: serum electrolytes (sodium, potassium, and chloride), serum albumin, serum urea and total protein. A total of 35 male wistar albino rats were weighed and randomly grouped into seven groups (five rats each). After the five days acclimatization, rats in Group 1, 2, 3 were orally administered aqueous extract of Glossocalyxbrevipes (B) leaf at 500mg/kg, 1000mg/kg and 2000mg/kg concentration respectively. Rats in Group 4, 5, 6 were orally fed the aqueous extract of Glossocalyxbrevipes (B) stem-bark at 500mg/kg, 1000mg/kg and 2000mg/kg respectively. Group 7 (control) was given 5ml/kg of distilled water.At the end of the twenty- one days experiment, blood samples were collected for biochemical assays. One-way analysis of variance (ANOVA) and least significant difference(LSD)test were used for data analysis. The results of the study shows that there was significant difference ($p<0.05$) in the serum levels of chloride (0.007mEq/L) ($p<0.05$), sodium (0.003mEq/L) ($p<0.05$) and albumin (0.348g/dl) ($p<0.05$) but there was no significant difference ($p<0.05$) in the serum levels of potassium(0.665mEq/L) ($p<0.05$), urea (0.874mmol/L) ($p<0.05$), total protein (0.503) ($p<0.05$). This study has therefore shown to some extent that aqueous stem-bark and leaf extract of Glossocalyxbrevipes(B) may not have significant effect on the kidney function of rats after a twenty-one day daily treatment. However, histological studies are needed to authenticate this claim.

Keywords: *Glossocalyxbrevipes* (B), rats, kidney function, serum electrolytes, albumin, urea, total protein.

INTRODUCTION

Vegetables are fresh and edible portion of herbaceous plants, which can be eaten raw or cooked [1]. They contain valuable food ingredients, which can be successfully utilized to build up and repair body tissues [2]. Many publications have emphasized on the diversity and value of traditional vegetables. The nutritional value of traditional leafy vegetables is higher than several known common vegetables [3]. Most of these traditional leafy vegetables have a potential for income generation but fail to compete with exotic vegetables at present due to lack of awareness[4]. Consumption of traditional diets known to these societies are said to have many beneficial effects such as prevention of some age related degenerative diseases – atherosclerosis, stroke, etc. [5]. Despite these advantages, most traditional plant foods are generally uncultivated and underutilized [6].

Vegetables are important sources of protective foods, which are highly beneficial for the maintenance of good health and prevention of diseases[7,8]. Some indigenous leafy vegetables grow in the wild and are readily available in the field as they do not require any formal cultivation. Many of them are resilient, adaptive, and tolerate adverse climatic conditions more than the exotic species [9]. Although they can be raised comparatively at lower management cost and on poor marginal soil, they have remained underutilized, due to lack of awareness of their nutritional values in favour of the exotic ones [10,11]. Leafy vegetables are rich sources of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorous [8].

The kidney is a major organ for metabolizing toxic compound besides liver. It receives about 1200ml blood per minutes containing a lot of chemical compounds [12]. Therefore high dose of *G.brevipes(B)* might cause damage to the kidney tissue which can be determined by measuring the level of urea and creatinine in blood as an indicator of kidney damage. The kidneys are vital excretory organs and are central to fluid, electrolyte and acid- base homeostasis in human [13]. Damage of the kidneys has serious implications for systematic functions, growth and existence. Irreversible damage that compromises the ability of the kidneys to sustain bodily function, normal growth and life as occurs in end stage renal diseases poses great challenges of renal replacement strategies and other management modalities [13].

Therefore the objective of this study is to determine the LD_{50} value of *G. brevipes (Benth),*determine the effect of the extracts on the serum electrolyte(sodium, potassium, chloride), total protein, albumin, urea levels, determine the optimal intake of the extracts.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The barks and leaves of *Glossocalyxbrevipes(B)* were collected from OmuookeEkiti. The leaves were identified as *Glossocalyxbrevipes (B)* at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Experimental Animals

Thirty male albino rats were purchased from the animal house of Department of Veterinary Medicine, University of Nigeria, Nsukka. They were weighed and kept in plastic cages with iron nettings in the animal house of Home Science, Nutrition and Dietetics. The animals were allowed to acclimatized for a period of 5 days and were fed with standard rat chow anddrink water *ad libitum.*

Preparation of *Glossocalyxbrevipes (B)* Sample

The stem bark was carefully removed, washed, cut into pieces, sun dried, milled or grounded with mortar and pestle and stored in labeled air tight containers. The leaves were picked, washed, cut and oven dried at 90°C for 6 h. The dried leaves were pulverized, package in airtight sterile bottles, labeled and stored in a refrigerator until used.

Preparation of the Aqueous Extract

The aqueous extract was obtained by adding 250mls of water into 247g of sample (leaves) and 200mls of water was added into 147.74g sample (bark) was kept in the refrigerator for 3 hours at 37˚c to the extract. The solution was filtered with a muslin cloth and extract stored in the refrigerator at 4^0C to avoid spoilage. Graded doses of the extracts (500, 1000, and 2000 mg/kg) were administered by oral gavage to each group of the animals. Water and food were given *ad libitum.*

Acute Toxicity and Lethality (LD_{50}) Test

Oral acute toxicity and lethality test (LD_{50}) of the aqueous extract was determined in mice as described by [14]. A total of 16 mice were used; three animals per group for each 10mg/kg, 100mg/kg and 1000mg/kg doses respectively and one animal for the control group took distilled water (5ml/kg). The lethal dose was calculated depending on the dose that was highly toxic to the animals.

Determination of Kidney Function Parameters

Chloride Determination

The Teco chloride reagent set for the quantitative colorimetric determination of chloride in human serum was used[15]. Clean test tubes were labeled blank, standard and sample respectively. About 1.5ml of chloride reagent was pipetted into each tube and 10µl each of different samples was added to respective tubes. They were incubated at

room temperature for at least five (5) minutes and absorbance read at 480nm using Unicamuv/vissible spectrophotometer.

Calculation

$$\text{Concentration of chloride} = \frac{\text{Abs of Unknown}}{\text{Abs of Standard}} \times \text{Conc. of calibrator (mEq/l)}$$

Total Protein (TP) Determination

Total protein concentrations (mg/ml) in the plasma were analysed with Follin-Ciocalteau reagent as described by Cunha-Bastos et al [16]. Bovine Serum Albumin (BSA) was used as standard protein.

Potassium Determination

Colourimetricdetermination of potassium in human and serum plasma according to Terry and Sesin [17].Test tubes were labeled: standard, sample and blank, 1.0ml of potassium Reagent was pipetted into test tubes and 0.01ml (10µl) of samples was added to respective tubes except blank. They were mixed and kept at room temperature for 3 minutes. After 3 minutes, the wavelength of spectrophotometer was set at 500nm and zeroed with reagent blank. The absorbance of all tubes were read and recorded.

Sodium Determination

Determination of sodium in human plasma according to the modified method of Trinder [18].The test tubes were labeled blank, standard, control and sample. About 1.0ml of Filtrate Reagent was pipetted into all tubes and 50 µl of sample added except for blank which received distilled water. The tubes were shaken vigorously for 3 minutes and centrifuged at high speed (1,500g) for 10 minutes; colour development was carried out by adding 1.0ml acid reagent to 50µl of the different supernatant and 50µl of colour Reagent added to all tubes and mix. The spectrophotometer was zeroed with distilled water at 550nm and the absorbance read.

Urea Determination

The Urease-Berthelot method for the quantitative *in vitro* determination of Urea in serum, plasma and urea was used. Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction [19].

Albumin Determination

The Bromocresol green (BCG) method for the quantitative *in vitro* determination of albumin in serum and plasma. The albumin-BCG-complex absorbs maximally at 578nm [20].

Statistical Analysis

Data was expressed as mean of standard deviation (± SD). The design of was a completely randomized design. The one-way analysis of variance (ANOVA) and least significant difference (LSD) were used for analysis of data ($P < 0.05$) to detect significance of the treatment of 5% probability level.

RESULTS

Acute toxicity studies

The LD_{50} of the leaf and bark extract was calculated to be greater than 5000mg/kg body weight. No physical symptom of toxicity was observed throughout the acute toxicity study period (Table 1).

The effects of the aqueous extract of *Glossocalyxbrevipes* (B) leaves and bark on the concentrations of some serum electrolytes are shown in Table 2. The leaf extract increased significantly ($p<0.05$) serum chloride ion concentration in 1000mg/kg GBLE (81.58mEq/L) followed by 2000mg/kg GBLE (72.86mEq/L), then by 500mg/k GBLE (62.11mEq/L) compared with the Control (34.39mEq/L).Similarly, the stem-bark extract of *Glossocalyxbrevipes*(B) increased significantly ($p<0.05$) the serum chloride ion concentration of 500mg/kg GBBE (58.24mEq/L) followed by 1000mg/kg GBBE (37.67mEq/L), 2000mg/kg GBBE (35.79mEq/L) that received highest dose whencompared with the Control (34.39mEq/L).

The effect of the *Glossocalyxbrevipes*(B) leaf extract increased significantly ($p<0.05$) the serum sodium ion concentration at 500mg/kg GBLE (141.90mEq/L) followed by 2000mg/kg GBLE (135mEq/L) when compared with the Control (105mEq/L). But there was a decrease in Na ion concentration at 1000mg/kg GBLE

(90.38mEq/L).Similarly, the bark extract of *Glossocalyxbrevipes* (B) had a significant increase ($p<0.05$) on the serum sodium ion concentration whencompared to the control group (Table 2).

The effect of *G.brevipes* (B) leaf extract did not have any significant effect ($p>0.05$) on the serum potassium ion concentration atany of the exposed concentrations when compared with the control. Similarly, the bark extract of *G.brevipes*(B) had no significant effect ($p>0.05$)on the serum potassium ion concentration art 500mg/kg GBBE (5.16mEq/L), 1000mg/kg GBBE (5mEq/L) and 2000mg/kg GBBE (5.33mEq/L) when compared to the control (4.46mEq/L).

The effects of the administration of the leaf and stem-bark extract on the total protein, serum albumin and serum urea concentrations are shown in Table 3. The *G.brevipes* (B) leaf extract had no significant effect ($p>0.05$) on the total protein of 500mg/kg GBLE (8.73g/dl), 1000mg/kg GBBE (14.23g/dl), and 2000mg/kg GBLE(8.15g/dl) when compared with the control (12g/dl).Similarly, the *G.brevipes*(B) bark extract had no significant effect ($p>0.05$) on the serum protein concentration when compared with the control.

G.brevipes (B) bark extract had no significant effect ($p>0.05$) on the serum urea concentration of the rats at 2000mg/kg GBLE (3.21mmol/L), 1000mg/kg GBLE (2.24mmol/L) and 500mg/kg GBLE (2.13mmol/L)compared with the control (2.03mmol/L).Similarly, the *G.brevipes*(B) bark extract had no significant effect ($p>0.05$) on the serum urea concentration of rats in 2000mg/kg GBBE had the highest value (2.52mmol/L), followed by 500mg/kg GBBE (2.18mmol/L) then by 2000mg/kg GBBE (1.98mmol/L) when compared with the control (2.03mmol/L).

The effect of *G.brevipes*(B) leaf extract on the result of serum albumin concentration shows significant increase ($p<0.05$) when compared with the Control. Similarly, the *G.brevipes*(B) bark extract has a significant effect ($p<0.05$) on the serum albumin concentration of rats in 1000mg/kg GBBE with the highest value (3.44g/dl), followed by 2000mg/kg GBBE (3.13g/dl) and lastly the 500mg/kg GBBE (2.79g/dl).

Table 1: LD_{50} value of the aqueous extract of *Glossocalyxbrevipes* (B) leaves and bark extracts

SN	No. of Mice	Dose	No. of death of animals
1	3	5mg/kg	0
2	3	50mg/kg	0
3	3	300mg/kg	0
4	3	2000mg/kg	0
5	3	5000mg/kg	0

LD_{50} value ≥ 5000 mg/kg, Animal used - albino mice, Weight of animals 20-25 g
No. of Animals – 3, Route – oral.

Table 2: Effects of*Glossocalyxbrevipes*(B) aqueous leaf and stem-bark extract on the concentrations of some serum electrolytes in Wistar albino rats

Group	Concentration of serum electrolytes (mEq/L)		
	Chloride	**Sodium**	**Potassium**
500mg/kg GBLE	62.11 ± 14.34	141.90±55.67	5.64±1.90
1000mg/kg GBLE	81.58± 27.35	90.38±18.06	6.36±2.09
2000mg/kg GBLE	72.86±27.81	135.00± 23.49	4.34± 1.79
500mg/kg GBBE	58.24± 29.36	114.60± 31.65	5.16± 1.05
1000mg GBBE	37.67± 19.79	65.70 ± 19.04	5.00± 2.09
2000mg/kg GBBE	35.79± 8.47	59.30±41.60	5.33±1.68
Control	34.39± 10.57*	105.00± 20.21*	4.46 ± 1.39
LSD	0.007	0.003	0.665

Decision rule: *if the difference between the two mean is greater or equal to the LSD value then the plants means are significantly different.*
500mg/kg GBLE =received 500mg/kg concentration of Glossocalyxbrevipes (B) leaf extract.
1000mg/kg GBLE = received 1000mg/kg concentration of Glossocalyxbrevipes (B) leaf extract.
2000mg/kg GBLE = received 2000mg/kg concentration of Glossocalyxbrevipes (B) leaf extract.
500mg/kg GBBE= received 500mg/kg concentration of Glossocalyxbrevipes (B) bark extract.
1000mg/kg GBBE= received 1000mg/kg concentration of Glossocalyxbrevipes (B) bark extract.
2000mg/kg GBBE= received 2000mg/kg concentration of Glossocalyxbrevipes (B) bark extract.
Control = received no extract.

Table 3: Effects of *Glossocalyxbrevipes* (B) aqueous leaf and stem-bark extract on some serum bio-molecules of male Wistar albino rats

Groups	Total protein g/dl	Urea mmol/L	Albumin g/dl
500mg/kg GBLE	8.73 ± 4.94	2.13± 0.72	3.92 ± 1.36
1000mg/kg GBLE	14.23± 11.75	2.24 ± 0.98	3.48 ± 1.77
2000mg/kg GBLE	8.15± 4.82	3.21± 1.98	3.37±0.38
500mg/kg GBBE	7.25 ± 5.12	2.18± 1.58	2.79± 0.54
1000mg/kg GBBE	15.04± 2.12	1.98± 1.72	3.44± 0.56
2000mg/kg GBBE	12.71± 6.28	2.52±1.29	3.13± 0.52
Control	12.00± 6.28	2.03 ± 1.12	2.57± 0.66
LSD	0.503	0.874	0.348

Decision rule: *if the difference between the two mean is greater or equal to the LSD value then the plants means are significantly different.*
500mg/kg GBLE =received 500mg/kg concentration of Glossocalyxbrevipes (B) leaf extract.
1000mg/kg GBLE = received 1000mg/kg concentration of Glossocalyxbrevipes (B) leaf extract.
2000mg/kg GBLE = received 2000mg/kg concentration of Glossocalyxbrevipes (B) leaf extract.
500mg/kg GBBE= received 500mg/kg concentration of Glossocalyxbrevipes (B) bark extract.
1000mg/kg GBBE= received 1000mg/kg concentration of Glossocalyxbrevipes (B) bark extract.
2000mg/kg GBBE= received 2000mg/kg concentration of Glossocalyxbrevipes (B) bark extract.
Control = received no extract.

DISCUSSION

The wide use of medicinal plants as food supplements, additives, spices and or for therapeutic purposes has formed the basis of nutrition and health care throughout the world, since the early days of humanity and has considerable importance [21]. Thus, knowledge of the use and side effects of these medicinal plants provide a vital contribution to human nutrition and health.These plants are relatively cheap and available and their uses are mostly dependent on past experience [22] and historical antecedents of local consumers. Majority of the population in the developing countries remain dependent on them for its nutrition and health benefits [23] without valid scientific knowledge of their toxicological effects. Therefore, the safety profile of these plants will justify their ultimate use by the general populace.

The lethal dose (LD_{50})result of the aqueous stem, bark and leave of *Glossocalyxbrevipes* (B) was found to be greater than 5000mg/kg suggesting safety of the plant in accordance with the recommendation of Organization for Economic Co-operation and Development (OECD). In addition, no physical symptoms of toxicity and death were noticed in the exposed mice used in this study. This agrees with the studies carried out on the kidney functions of rats treated with *Ziziphusmucronata* [24] which also showed no symptoms of toxicity and death.

From the result of the kidney function test, three of the kidney function parameters analyzed had a significant change ($p<0.05$) while the other parameters were not significant. The serum urea, protein and albumin usually determine the general function of the kidney while the electrolytes are determinants of tubular function [25].

Urea is a by- product from protein breakdown. About 90% of urea produced is excreted through the kidney [26]. Therefore, high level of blood urea and Creatinine will indicate kidney damage. In this study, the serum urea which was not statistically different from the control group suggests that the aqueous extract of *Glossocalyxbrevipes* (B) was not nephro-toxic in rats within the study period. The same observation was made by Al-Ameen, *et al*., [27] in normal rats treated with aqueous extract of (*Nigella sativa).*

The total protein test measures the total amount of two classes of protein found in the fluid portion of the blood; these are the albumin and globulin [28].The result of this study showed that the total protein analyzed in rats were not statistically different from the control group, indicating that the aqueous leaf and bark extract of *Glossocalyxbrevipes* (B) was not toxic to the kidney. Proteins circulate throughout the blood to help maintain body fluid balance. Albumin is produced in the liver and is one of the most abundant proteins in the blood fluids or plasma. A proper balance of albumin is required to keep the fluid from leaking out of blood vessels. Albumin also carries vital nutrients, hormones and proteins required to clot blood properly [29]. The typical value for serum albumin is 3.4 to 5.4g/dl of blood according to National Institute of Health (NIH). The aqueous extract of *Glosscalyxbrevipes* (B) leaf and stem-bark increased the albumin levels of rats, within the normal range.

Chloride levels usually fluctuate with sodium levels. It works with other electrolytes such as potassium, sodium and carbon dioxide (CO_2). High chloride levels may result from dehydration but can also occur with other problems that cause high blood sodium such as kidney disease [30]. In order wards, the result showed a significant change in the serum chloride levels of rats, but the increase and decrease in the values were all within the normal range. Sodium is regulated by the kidneys and adrenal glands [28]. The results of this study showed a significant increase in the sodium levels of rats but the difference in the various doses were within the normal range. The observations made in this study is not similar to that made by [25] in normal rats treated with aqueous extract of (*Ziziphusmucronata)* which had no significant effect on the sodium levels of rats.

Potassium levels often change with sodium levels. When sodium levels goes up, potassium levels goes down, and *vice versa* [30]. In this study, there was no significant increase on the potassium levels of rats. This was similar to the observation made by [27] on rats given the aqueous extracts of (*Nigella sativa)* which had no significant effect on the potassium levels of rats.

This study also found that the body weights of the rats in all groups increased as well as the quantity of food consumed during the experimental period although no behavioral signs of an increased appetite were indicated. This may be that the leaf and stem-bark extract may be responsible for boosting the appetite or may be as a result of other unforeseen factors. This is a positive advantage derived from the consumption of *G. brevipes*.

Traditionally the local consumers use the bark pulp in soup preparations but this study shows that the leaf extract gave better biochemical responses than the bark. We therefore suggest that the leaves of *brevipes* may be used as soup condiment especially in rainy season during which seeds from these plant grow.

In conclusion, evidence of normal electrolytes, albumin, urea and total protein in the blood samples of all treatment groups suggest that there was no toxic effect of *Glossocalyxbrevipes* (B) on the kidney functions of the Wistar albino rats at different doses during the twenty one day period of exposure. Therefore, consumption of this indigenous vegetable could be used for its nutritional and therapeutic purposes.

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