SUB-ACUTE AND SUB-CHRONIC TOXICITY STUDIES ON LEAF EXTRACTS OF FLUTED PUMPKIN (*Telfairia occidentalis*) USING WISTAR RATS

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ABSTRACT: Fluted pumpkin (*Telfairia occidentalis*) is a popular tropical vine grown mainly in West Africa for its abundant nutritional and medicinal potentials. The present study was carried out to evaluate the possible toxicity effect of the plant extracts in normal rats. The design consisted of sixteen rats divided into 4 groups of 4 rats in each group. Three of the groups respectively received 125, 250 and 500mg/kg body weight of leaf extracts for 21 days and the remainder served as control which received placebo treatment. Body weight measurements and behavioral changes were taken during this period. Hematological parameters and biochemical indices of liver and kidney function were respectively evaluated in whole blood and serum collected from the animals after the 21-day treatment. Result of the measured indices indicated no statistically significant changes in behavior, body weight, hematological and biochemical indices of the test animals relative to the control group ($p>0.05$). These preliminary toxicological results suggest that sub-acute and sub-chronic administration of rat with ethanolic leaf extracts of *Telfairia occidentalis* may have no severe toxicological consequences.

INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis*) is a tropical vine grown mainly in West Africa for its vegetable (Akoroda, 1990). It is also known as Fluted goard. In Nigeria, it is known locally as Ubong by Ibibios, Ugu by Ibos and Iroko by Yorubas ethnic groups (Abiose, 1999). The Spanish called it Albaza Costillada, the Ghananians refers to it as okrobonka while to the Siera Leoneans, it is known as Oroko (Abiose, 1999). The plant is tolerant to drought. It is dioeciously perennial and usually grown trellised. The young shoots, seeds and leaves of the female plant are the edible parts. The roots of the plant are said to be poisonous (Abiose, 1999).

The fluted pumpkin has remained a regional treasure in West Africa (Abiose, 1999). It is widely employed for its medicinal and nutritional potentials. It is popularly used in Ethnobotanical as antidiabetic, antihypertensive, antitumoric, antioxidant, immunodulator, antibacterial, antihypercholesterolemic, intestinal antiparasite and anti-inflammatory agent (Nwozo *et al*., 2004). Considerable evidence from several epidemiological studies concerning the use of its bioactive substance on a number of animal model, cell culture studies and clinical trials validates its immense pharmacological activities (Sofowora, 1996; Gbile, 1986; Odoemena and Essien 1995; Eseyin *et al*., 2000, 2005; Nwozo *et al*., 2004; Oboh *et al*., 2006). This has stimulated research interest on this plant (Schippers, 2000) as can be seen by the upsurge of literatures. Its succulent tasty leaves, stems and seeds make fluted pumpkin one of the widely eaten vegetables in homes and in restaurants across West Africa (Abiose, 1999). Young shoots and leaves are used for making soups for different kind of starchy dough and may be cooked alone or in mixture with other vegetables. Immature seeds are usually preferred to mature ones and are eaten cooked or roasted (Akwaowo *et al*., 2000). Seed cotyledons are processed into seasonings and are rich in protein content therefore, are used for infant weaning foods, flour bread supplement and different local fermented foods (Egbekun *et al*., 1998; Giami and Isichei, 1999; Giami *et al*., 2003). The seeds are reported to have lactation – promoting

properties and are highly used by nursing mothers (Schippers, 2000). Mature seeds are good source of edible unsaturated oil (Odoemena and Onyeneke, 1988; 2000; Giami *et al*., 1999). The economic and nutritional value of the plant in West Africa can therefore not be overemphasized as it features prominently in trans-border trade especially among Nigeria, Cameroon and Benin Republic (Giami *et al*., 2003).

Studies conducted on this plant are mainly nutritional and medicinal. However, a necessary component of any nutritional and medicinal potentials of an herb is toxicity studies and there is paucity of information with respect to *Telfairia occidentalis*. The present study evaluated the toxicity consequence of extracts from leaves of *Telfairia occidentalis* administered at sub-acute and sub-chronic levels, using hematological, liver and kidney function indices as marker parameters in Albino Wistar rats. The immense nutritional and medicinal benefits of the plant have the tendency of increasing the usage of this plant as food, food supplement or its bioactive compounds in the development of novelty drugs. In order to guarantee its safety, an essential step is to evaluate its toxicity. Therefore this study is significant because it will help to predict the hazard of long-term exposure as well as evaluate the safety level of administration.

MATERIAS AND METHOD

Fresh immature leaves of fluted pumpkin (*Telfairia occidentalis*) were purchased at Watt Market, Calabar. The leaves were authenticated by a staff in the herbarium of the Department of Botany, University of Calabar, Calabar, Cross River State. They were then brought to the Endocrine Research Laboratory, University of Calabar where they were selected to remove extraneous materials, immediately washed with distilled water and drained. The leaves were then chopped into small pieces and weighed at 50g each time to obtain a total of 1150g. The measured leaves were blended using an electric blender with appropriate amount of ethanol (i.e. 2.0L of 80% ethanol (BDH) stored for 48 hours in the lower apartment of a refrigerator between 0-8^{0}C. It was then filtered using cheese cloth, beaker and funnel to obtain extract which was further concentrated using rotary evaporator and a water bath at a temperature not exceeding 45-50^{0}C. Test extracts were re-dissolved in dimethyl sulfoxide (DMSO).

Sixteen (16) disease free stock Albino Wistar rats of both sexes weighing between 110-200g were obtained from the animal house of the Department of Biochemistry, University of Calabar, and used for the study. These animals were housed in plastic cages (North Kent co Ltd) in the Endocrine Research Laboratory, University of Calabar, throughout the duration of the research which lasted for 21 days. The animals were acclimatized to the laboratory environment for a period of two weeks. After the period of acclimatization, they were randomly assigned to four (4) groups with 4 rats in each group. Three groups of the animals served as experimental test groups T_1, T_2 and T_3 while one group (Co) served as the control. The three test group received the leaf extract which was re-dissolved in dimethyl sulfoxide (DMSO) at doses of 125mg/kg, 250mg/kg and 500mg/kg body weight respectively. The control group received dimethyl sulfoxide (DMSO) only. Animals were kept within the same condition of temperature, relative humidity and Lighting (12 hours light and dark cycle). Food (Livestock grower) and water (clean tap water) were provided *ad-libitum*. Test substances were administered orally with the aid of a cannula. The initial body weights of the animals in each group were noted.

During the duration of the experiment, the animals were observed for behavioural display such as muscular weakness, salivation, diarrhea, changes of colour of hair, flaking of hair and food intake for 15 minutes before the administration, 5 minutes for drug stability and then for the next 40 minutes. At the end of the duration of the experiment, the final body weights in each group were noted. The animals were kept on an overnight fast and were anaesthesized the following morning using chloroform vapour. They were placed on a dissecting slab. A longitudinal cut was first made abdominally to the rib cage followed by transverse cut to the limbs. Blood was collected through cardiac puncture, using sterile needle to piece through the heart. Blood samples were collected for whole blood and for serum. Samples collected for

whole blood were emptied into a sterile test tube containing EDTA anticoagulant. The whole blood samples were carefully handled to avoid hemolysis of the cells and were used for the estimation of hematological indices.

Blood samples collected for serum were put in a sterile test tube containing no anticoagulant and were allowed to clot and centrifuged at 3000 rpm for 10 minutes using MSE table top centrifuge. Serum was collected using syringes into labeled specimen tubes and used for the estimation of the liver and kidney function indices. The kidney and liver were dissected out, washed and transferred into an ice-cold saline solution. They were homogenized in 0.1m tris HCL buffer at pH 7.4, and the homogenates were also analyzed for the liver and kidney function indices.

Total count of white blood cells (WBC) and red blood cells (RBC) were determined by the methods of Kolmer *et al*, (1951). Packed cell volume (PVC), Hemoglobin and Platelets (PL) concentrations were determined using standardized laboratory methods (Automated hematology Analyser). Aspartate transaminase was determined using Randox Reitman Kit, Alanine transaminase by Reitman and Frankel (1957) and Alanine phosphatase by Tietz kit (Tietz, 1976). Urea concentration was determined by the methods of Searcry *et al*., (1967); creatinine by the methods described by Newman and Price (1999); Potassium ion and chloride ion by colorimetric procedures.

The arithmetic mean ± SD of the data were determined and then analysed statistically using students't-test. A probability value of 95% was taken to indicate a statistical significant difference between the two groups compared.

RESULTS AND DISCUSSION

Table 1 shows the mean body weights of animal before and after the 21 days of treatment. The body weight gains were 27.80±23.57gm; 27.90±54gm; 29.95±15.16gm and 24.63±16.53gm for the control, low dose, medium and high doses respectively. However, this apparent weight gain difference was not statistically significant ($P<0.05$). Changes in body weight are used as an indicator of adverse effect of drugs or chemicals (Tofovie and Jackson, 1999; Raza *et al*., 2002). In this study, treatment resulted in small, albeit insignificant weight gains suggesting that ethanolic extract is non-toxic in experimental rats. No muscular hypotrophy was observed either in the hind or fore legs. Daily food intake was normal and there was no diarrhea or excessive salivation. The hair colouration did not change nor did the hairs flaks off abnormally. These observations further support that the ethanolic leaf extracts is non-toxic to rats.

Table 1: Effect of ethanolic leaf extract of fluted pumpkin (*Telfairia occidentalis*) on Growth Performances in Rats.

Parameters/unit	Co = Control	T_1 = Low dose 125mg/kg	T_2 = Medium dose 250mg/kg	T_3 = High dose 500mg/kg
Initial Body Weight (gm)	146.03±50.22	157.90±70.1	148.35±37.67	131.03±19.54
Final Body Weight (gm)	173.83±14.83	185.75±55.5	178.15±25.80	155.67±38.67
Body Weight Gain (gm)	27.80±23.47	27.90±54.58	29.95±15.16	24.63±16.53
Mortality (%)	25	50	0	25

The hematological profile of the experimental and control groups of the rats are shown in Table 2. No significant changes in the values of RBC, WBC PVC and platelets of the experimental animals were observed when compared to the control group except for the hemoglobin concentration which tended to increase significantly ($P<0.05$) as the concentration of the extract administered increases. This could be as a result of the high content of the leaf extract. Data on mineral element estimation of the leaf of *Telfaira occidentallis* showed that the leaf contained about 0.992g/kgDM of iron and was found to be higher than the level in most common vegetables (Alada, 2000; Akwaowo *et al*., 2000 and Fasuyi, 2005,).

Since the administration of the ethanolic leaf extract did not alter the hematological profile of the rat, it is obvious that treatment has no adverse effect or are non-toxic to experimental rats.

Tables 3 and 4 show the sub-chronic toxicity effect of treatment on the liver and kidney function indices in rats. When there is acute damage to the liver, some of its enzymes are liberated into blood, where they can be measured. The commonest enzymes employed as indicators of hepatocellular damage are the transaminases: aspartate aminotransferases (AST), and alanine amino transferases (ALT) as well as alkaline phosphatase (ALP). Increases in serum level of these enzymes are routinely determined to evaluate the extent of liver damage (Gaw *et al*., 1995). Elevations in the levels of aminotransferases are frequently the first findings during hepatocellular damage due to drugs or other toxic agents (Gaw *et al*., 1995). These enzymes are released from hepatic cells when there is damage or increased cell permeability. Serum enzyme levels are particularly high in acute hepatocellular damage, chronic hepatocellular disease and in cholestatic conditions.

Table 2: Effect of ethanolic leaf extract of fluted pumpkin (*Telfairia occidentalis*) on haematoligcal indices in rats.

Parameters	Control	Low dose 125mg/kg	Medium dose 250mg/kg	High dose 500mg/kg
WBC ($x10^9$/L)	9.5±3.0[a]	9.5±1.9[a]	8.8±1.6[a]	8.3±1.8[a]
HGB (g/l)	133±15.6[a]	147±12.1[b]	138±9.9[a]	271±12.1[b]
RBC ($x10^9$/L)	7.1±1.0[a]	8.1±0.4[a]	7.5±0.7[a]	6.95±2.1[a]
PVC (%)	44.9±5.9[a]	49.8±1.98[a]	48.1±2.1[a]	46.6±2.1[a]
PLT (x 10^9/L	668.3±194.8[a]	657.5±280.7[a]	697±94.8[a]	608±174.0[a]

WBC = White blood cell; HGB = Hemoglobin; RBC = Red blood cell; PVC = Packed cell volume, PLT = Plateletes.

Table 3: Effect of ethanolic leaf extract of fluted pumpkin (*Telfairia occidentalis*) on liver function indices in rats.

Parameters	Co = control	T_1 = low dose 125mg/kg	T_2 = medium dose 250mg/kg	T_3 = high dose 500mg/kg
Alkaline phosphatase	12.11±32.35[a]	162.62±25.92[a]	110.72±31.65[a]	311.34±91.47[a]
Aspartase transaminase	15.00±1.41[a]	17.0±4.24[a]	14.75±3.30[a]	15.00±1.00[a]
Alanine transaminase	48.00±0.00[a]	57.0±0.00[a]	48.00±0.00[a]	57.00±0.00[a]

Table 4: Effect of ethanolic Leaf extract of fluted pumpkin (*Telfairia occidentalis*) on kidney function indices in rats.

Parameters/unit	Co = Control	T_1 = Low dose 125mg/kg	T_2 = Medium dose 250mg/kg	T_3 = High dose 500mg/kg
Urea (mg/dl)	23.73±8.3[a]	22.95±21.99[a]	37.88±26.65[a]	32.6±7.64[a]
Creatinine (mg/dl)	2.08±0.23[a]	1.62±0.28[a]	2.32±0.26[a]	1.83±0.20[a]
Potassium ion (mmol/L)	8.25±0.49[a]	11.5±1.13[a]	8.48±2.09[a]	8.03±2.59[a]
Chloride ion (mmol/L)	99.60±31.31[a]	104.95±14.26[a]	108.25±12.48[a]	144.64±21.55[a]

The result in Table 3 shows that there is no significant mean difference ($P<0.05$) between the treatment groups and the control. Therefore, sub-chronic administration of ethanolic leaf extract of *Telfairia occidentalis* is not hepatotoxic. Eseyin *et al*., 2005, reported that the ethanolic leaf extract of *Telfaira occidentalis* may rather be beneficial in the management of cholesterolemia, liver problems and impaired immune systems. Its antioxidant capabilities were earlier demonstrated by Ajibesin *et al*., (2002), who reported that the vegetable helps to restore damage to liver cells, protect the heart and enhance youthfulness. It was also reported to have protective role against liver damage caused by agent like Paracetamol and other toxins.

Table 4 shows the effect of treatment on the kidney function indices in Rats. There was no significant difference ($P<0.05$) in the urea, creatinine, potassium and chloride ion concentrations of the treatment group compared to the control. The chloride ion concentration tended to increase as the extract concentration administered increases. At the concentration of 500mgkg body weight, a very high increase in the chloride ion concentration was noticed. The reason for the increase in the chloride concentration is not known but we suspect that it might be due to hemolysis. Examination of the red blood cells count in the high dose level 500mg/kg $-T_3$, showed that it was $6.95\pm2.1 \times 10^9$/L and this was lower than any of the other treatment groups and the control ($7.1\pm1.0 \times 10^9$/L), though not statistically significant ($P<0.05$), but this perhaps signifies that a massive destruction of red blood cells took place, which probably could have resulted in concomitant increase in chloride ion concentration.

CONCLUSION

Our data suggest that sub-chronic treatment of rats with ethanolic leaf extract of fluted pumpkin (*Telfairia occidentalis*) has no harmful effects. Long term toxicity studies are suggested in order to rule out any long-term adverse effect.

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