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Effect of the root extract of *Telfairia occidentalis* on some biochemical parameters in rat

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Abstract
The effect of the ethanol root extract of *Telfairia occidentalis* on glucose level was evaluated at different dose levels in normoglycaemic rats (150,250, and 500mg/kg), glucose loaded rats (150 and 500mg/kg), and sub acute administration of 200mg/kg of the extract for 15 days. Blood glucose was determined at 0, 1, 2 and 4 hours; 0,15,30,45 and 60 minutes in the normoglycaemic and glucose loaded rats, respectively. In the sub acute administration of the extract, blood glucose level was estimated on days 1, 5, 9 and 15 with weights of the rats taken every other day. On the 15th day of sub acute administration, the rats were sacrificed and blood collected from their hearts. Aspartate and alanine aminotransferases, alkaline phosphatase, cholesterol, triglycerides, high density-, low density-, very low density-lipoproteins, glucose, creatinine and total proteins. The extract did not show any hypoglycaemic effect. And none of the parameters evaluated in the test animal showed significant difference from the control. The results show that, unlike the leaf, the root of *T. occidentalis* did not possess hypoglycaemic activity and the claim of toxicity of the root when eaten was not confirmed by this work.

Keywords: *Telfairia occidentalis*; Glucose; Toxicity; biomolecules

Introduction

Telfairia occidentalis Hooker F (Cucurbitaceae) is popularly known as fluted pumpkin and is widely cultivated in the southern part of Nigeria. Its nutritional value is well known and documented (Bosa *et al.*, 1983; Okigbo, 1997; Johnson *et al.*, 1979; Jeffrey, 1980; Burkill, 1979 and Akoroda, 1990). It is also reputed to be medicinally useful (Kafaru, 1998). Some of the medicinal values of the plant have been established by various researchers. It possesses hypoglycaemic activity (Eseyin *et al.*, 2000 and 2005a; Aderibigbe *et al.*, 1999; Nwozo *et al.*, 2005); anti inflammatory effect (Oluwole *et al.*,2003), erythropoietic value (Ajayi *et al.*,2000).The leaf extract have been found to be useful in the treatment of cholesterolemia, liver problems and impaired immune/defence system in rat (Eseyin *et al.*, 2005b).

It is generally believed that the root of *T. occidentalis* is poisonous (Smith *et al.*, 1997; Iwu, 1986) and could be rodenticide, fish and rat poisons (Sofowora, 1986; Hutchinson *et al.*, 1958). The root has been shown to possess antibacterial activity (Odoemena *et al.*, 1995).

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This work was aimed at determining whether the root extract of the plant possessed hypoglycaemic effect like the leaf and to identify some of the biomolecules affected by its activity,

Experimental

Collection and extraction of the roots. The roots of *T. occidentalis* were collected in September 2004 from Itak Ikot Akap in Ikono Local Government Area of Akwa Ibom State and identified at the Pharmacognosy Department, University of Uyo. They were washed with water, cut into smaller pieces and sun-dried. The dried roots were pulverized with a manual grinding machine. 350g of the powdered root was exhaustively extracted with 3.7litres of 96% ethanol in a Soxhlet apparatus. The extract was concentrated *in vacuo* and the dark-brown residue obtained was dried in a desiccator.

Animals. Wistar albino rats were purchased from the animal house of the Biochemistry Department of the University of Calabar. The animals were transferred to and kept in the animal house of the University of Uyo. They were given free access to food (Growers palletized feed from Vital Feeds, Jos, Plateau State) and water. They were kept under standard conditions in the care of qualified and experienced animal technicians.

Administration of extract to normoglycaemic rats. Twenty overnight fasted rats were divided into four groups (A, B, C and D) of five rats each and were given a single oral dose of 150,250,500mg/kg of the extract and distilled water only, respectively.

Administration of extract to glucose loaded rats: Fifteen overnight fasted rats were divided into three groups of five rats each. All the rats were given a single dose of 1g/kg glucose and extract simultaneously: Groups A, B, and C received 150 and 500mg/kg of the extract, and distilled water, respectively.

Sub acute administration of extract. A single dose of 200mg/kg of the extract was orally administered daily to 5 rats for 15 days. 5 other rats which served as the control received distilled water instead of the extract.

Determination of blood glucose level. Blood was obtained from the tail vein of the rats at 0, 1, 2 and 4hours; 15, 30, 45 and 60minutes; day0, 5, 10 and 15 in the normoglycaemic, glucose loaded, and sub acute extract administration, respectively. Blood glucose level was estimated using One Touch R Glucometer (Lifescan, Inc. 1995 Milpas, California 95035).

Effect of sub acute administration of extract on some biomolecules: At the end of the 15days daily administration of 200mg/kg extract, the rats were anaesthetized with chloroform and dissected. Blood was collected from the heart of the rats. The blood collected was centrifuged at 700rpm for 10minutes to obtain the serum. The serum was then stored at –4C until when used for analyses.

Estimation of biomolecules; The serum was analysed for the following enzymes and biomolecules, using appropriate kits(Randox Lab., U.K.): Alanine and aspartate aminotransaminases, alkaline phosphatase, total cholesterol, total proteins, triglycerides, creatinine, high-, low-, very low-density lipoproteins.

Glucose: This was evaluated using the glucose oxidase method (Trinder, 1969).

Alanine Transaminase (ALAT): The method involves the monitoring of the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine (Rietman and Frankel, 1957).

Aspartate aminotransferase (ASAT): The principle of the method used involved monitoring the concentration of oxaloacetate hydrazone formed with 2,4,-dinitrophenyl hydrazine (Rietman and Frankel, 1957).

Alkaline phosphatase (Phenolphthalein monophoshate method): This method is based on the principle that serum alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein that results in phosphoric acid and phenolphthalein at alkaline pH values. The

pinkly coloured product is measured colorimetrically at 550nm.

Triglycerides: This involves the enzymatic colorimetric test of glycerol phosphate oxidase method (Zoppi and Fenili, 1976) .

Total Cholesterol: This was carried out by the enzymatic colorimetric chod-PAP method (Zoppi and Fenili, 1976).

HDL-Cholesterol: high density lipoprotein (HDL) separated from chylomicrons. Very low density lipoproteins (VLDL) and low density lipoproteins (LSL) by the addition of a phosphotungstic and magnesium chloride (precipitating reagent) to the serum. After centrifugation, the cholesterol content was determined by the enzymatic colorimetric method (Zoppi and Fenili, 1976).

LDL and VLDL Cholesterol: These were calculated as recommended by Tietz (1999).

Total Protein: This was done using the Biuret method.

Creatinine: Modified Jaffe's method was used. Creatinine which is a hydride of creatine reacts with alkaline sodium picrate to form a red complex which can be determined photometrically (Jaffe, 1886).

Statistical analysis: Data were expressed as Mean ± SEM and were analysed by two-way ANOVA and Scheffe's post test. $P<0.05$ was taken as significant.

Results and Discussion

The results obtained are shown in Tables 1-5.The figures in parenthesis in Tables 1-3 are percent change in glucose (G).

$$G = Gt \times 100/Go$$

Where Go and Gt are glucose levels at time 0 and t, respectively. It is obvious from the results that the ethanol root extract of *T. occidentalis* did not exhibit any hypoglycaemic effect in the normoglycaemic (Table 1), glucose loaded (Table 2) and sub acute (Table 3) states at any of the dose levels. Rather, the extract surprisingly increased blood glucose level significantly (P 0.05) in both the normoglycaemic rats (at 1 and 2 hours). This is contrary to the observed hypoglycaemic effect of the leaf (Eseyin *et al.*, 2005b).

There was also no significant difference between the test and control groups in the serum levels of the enzymes and biomolecules estimated after daily oral administration of 200mg/kg extract for 15days (Table 5). Animals in both groups showed similar growth pattern as observed from their appearance and weights (Table 4).

Also, the $_{LD50}$ value of 1000mg/kg reported by Ajibesin *et al.* (2002) for the orally administered root extract seem to be high as to justify the popularly held belief of toxicity based on the classification of the British Toxicity Society Working Party on Toxicity (1984). Although Smith *et al.* (1997) have established the toxicity of the aqueous root extract when administered intraperitoneally, the administration of the ethanol extract through the oral route does not seem to pose this danger. The toxic constituents are probably either not concentrated in the ethanol extract as much as in the aqueous extract, poorly absorbed into the blood or are destroyed in the gastrointestinal tract.

Table 1: Effect of the root extract of *T. occidentalis* on the blood glucose level (mg/kg) of normoglycaemic rats.

Dose (mg/kg)	0 hour	1 hour	2 hours	4 hours
150	40.8 ±2.03 (100)	50.6±0.34 (124.1)*	43.6±1.33 (106.9)*	39.6 ± 5.33 (96.7)
250	47.0 ± 4.90 (100)	48.4±4.04 (112.8)*	40.8 ±1.99 (94.8)	41.0 ± 2.09 (95.6)
500	41.2 ± 3.84 (100)	47.8± 3.86 (116.2)*	49.6 ±4.12 (120.8)*	37.6 ± 4.12 (91.7)
Control	42.8 ± 5.49 (100)	41.0 ± 2.60 (96.7)	38.2±2.45 (90.8)	34.4 ± 4.71 (88.7)

Mean ± SEM n=5 * $p < 0.05$

Table 2: Effect of the root extract of *T. occidentalis* on blood glucose level (mmol/L) of glucose loaded rats (1g/kg)

Dose (mg/kg)	0 min	15 min	30 min	45 min	60 min
250	2.43±0.28 (100)	7.8 ± 3.15 (326.2)	14.47±8.32 (598.8)*	8.73±4.21 (366.2)	5.35±2.28 (227.7)
150	2.88±0.13 (100)	6.45±1.42 (225.8)	7.08 ±1.03 (247.9)	5.55±0.58 (194.2)	2.40±0.37 (84.0)
Control	3.05±0.27 (100)	7.30±2.06 (237.8)	6.95 ±1.82 (232.8)	7.15±2.37 (268.4)	4.55±2.75 (159.2)

Mean ± SEM n=5 * p 0.05

Table 3: Effect of the sub acute administration of the root extract (200mg/kg) of *T. occidentalis* on the blood glucose level (mmol./kg) in rat.

Day→	1	5	9	15
Test	3.763 ±0.41(100)	3.596 ± 0.97(95.56)	4.04 ±1.02(107.36)	8.78 ± 2.69(233.32)
Control	3.175 ± 0.79(100)	2.309 ±1.16(72.72)	3.937 ± 2.06(124.0)	7.58 ±1.69(238.74)

Mean ± SEM n= 5

Table 4: Effect of the sub acute administration of the root of *T. occidentalis* (200mg/kg) in the serum levels of some biomolecules

S/No	Biomolecule	Test	Control
1	Aspartate transaminase (U/L)	21.70±14.41	18.40 ±3.61
2	Alanine transaminase (U/L)	9.80± 2.87	12.20 ±5.34
3	Alkaline transaminase(U/L)	33.79 ±1.90	36.23 ± 1.65
4	Cholesterol (mmol/L)	5.87± 0.30	5.89± 0.52
5	Triglycerides(mmol./L)	2.36 ± 0.33	2.41 ± 1.10
6	High density lipoproteins (mmol/L)	1.06 ± 0.06	0.92 ±0.09
7	Low density lipoproteins (mmol/L)	5.25 ± 0.31	5.45 ± 0.62
8	Very low density lipoproteins (mmol/L)	0.47 ± 0.07	0.48 ± 0.62
9	Glucose (mmol/L)	8.76 ± 2.69	7.58 ±1.69
10	Creatinine (mmol/L)	109.82±10.21	101.37±23.93
11	Total proteins (g/L)	81.03 ±14.61	88.39 ±1.69

Mean ± SEM n=5

Table 5: Effect of the sub acute administration of root extract of *T. occidentalis* on weight (%) of rats

Day	1	3	5	7	9	11	13	15
Test	100	107.03	110.69	114.47	118.87	125.20	134.28	139.53
Control	100	105.18	111.20	113.50	117.20	123.48	132.56	139.01

It thus appears that the root may not be as poisonous as claimed (Sofowora, 1986; Smith *et al.*, 1997) when eaten. Further work is hereby suggested on this.

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