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Effect of Sub acute Exposure to *Telfairia occidentalis* Root, Pod and Stem Extracts on Some Liver and Renal Function Parameters in Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author EAO designed and carried out literature searches, experimental process and compiled manuscript. Author POU supervised research design and experimentation and revised the initial manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study assessed the effect of twenty eight (28)-day exposure to aqueous extract of *Telfairia occidentalis* root, pod and stem on the liver and kidney of Wistar rats.
Methodology: Sixty eight (68) wistar rats were distributed into 17 groups of 4 animals per group and administered, per os (p.o), distilled water, root, pod, and stem extracts each at 250, 750, 1500, 2250, and 3000 mg/kg body weight (bw). All animals were treated for 27 days, followed by sacrificing under chloroform anesthesia on the 28th day.
Results: Biochemical assay results showed that the extracts had varying effects on the liver and kidney and these effects do not correlate directly with dose administered. The root extract showed

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some degrees of hepatotoxicity and nephrotoxicity at half the maximum dose, and no toxicity observed at maximum dose. The pod extract caused disruption in liver membrane integrity and hyperalbuminemia, while the stem extract caused no necrotic injury but hypoalbuminemia. Both extracts showed mild to significant renal toxicity.

Conclusion: The non correlation of dose and effect was suggested to be due to different concentrations of the inherent bioactive principles which may be cumulative; exhibiting some synergistic and /or antagonistic tendencies, either which is achievable with varying dose and duration.

Keywords: Telfairia occidentalis; root; pod; stem; hepatotoxicity; nephrotoxicity; hyperalbulminemia; hypoalbuminemia.

1. INTRODUCTION

In a world where close to 600 million people live below poverty line, endangered by hunger and sickness [1,2], alternative sources of food and medicine is very necessary. Some by-product of food processing can step into this role as they are cheaper and available. But safety is of essence here as such by-products may be poisonous and may contain anti-nutritional factors.

Telfairia occidentalis (Fluted Pumpkin) is a common vegetable plant that is cultivated in both subsistence and commercial quantity in Nigeria, West Africa. It belongs to the tribe, Joliffieae and sub family, Cucurbitaceae, and the leaf and seed are consumed locally and in other sub Saharan Africa as food vegetable [3]. The nutritional and antioxidant potentials of the leaves of *T. occidentalis* has been extensively reported [4-6]. The leaves have also been reported to possess antiplasmodial, antimicrobial, and antidiabetic properties [7-10]. Apart from the leaf and the seed, which is edible, other parts of the plant are neither considered for nutritional nor medicinal importance and therefore discarded [11].

Our aim was to ascertain the effect of sub acute exposure to extracts of root, pod and stem of *T. occidentalis* on some biochemical markers of liver and renal function in experimental rats.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals and reagents used in this study were of analytical grade and product of Sigma-Aldrich Laborchemikalien and BDH Laboratory, Poole England. Commercial reagent kits (Randox, UK) were used for estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH).

2.2 Plant

T. occidentalis root, pod and stem were collected from a farmland in Port Harcourt, Nigeria. Samples were identified at the Herbarium of Plant Science and Biotechnology Dept. of University of Port Harcourt. The samples were washed, cut into smaller bits and dried under shade.

The plant samples were powdered and each sample (600 g) was macerated in adequate aqueous solvent for 24 hours to obtain crude extracts. The extract solutions were filtered and concentrated at 40℃. The extracts were stored in the refrigerator until used.

2.3 Animals

Sixty eight (68) Wistar rats were obtained from University of Nigeria, Enugu Campus and kept in the animal house of Biochemistry Dept, University of Port Harcourt. They were acclimatized for two (2) weeks with unlimited access to water and normal rat chow.

Wistar rats of both sexes were assigned to 17 groups of 4 animals per group. Different groups received distilled water (2 groups), root, stem, and pod extracts at the doses of 250, 750, 1500, 2250, and 3000 mg/kg of body weight, p.o. All animals were treated for 27 days, followed by sacrificing under mild chloroform anesthesia on the 28th day.

2.4 Biochemical Analysis

2.4.1 Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

ALT and AST activities in the serum were determined by colorimetric method of Reitman and Frankel [12].

2.4.2 Serum alkaline phosphatase (ALP)

ALP activity was assayed by the method of Kind and King [13].

2.4.3 Serum lactate dehydrogenase (LDH)

LDH activity in the serum was estimated by UV method based on Rec GSCC [14].

2.4.4 Total protein

Total protein assay was based on Biuret method as described by George and O'Neill [15].

2.4.5 Serum albumin

Determination of serum albumin was based on bromocresol green (BCG) method as described by Tietz [16].

2.4.6 Serum urea

Estimation of urea was carried out by diacetyl monoxime method as described by Rosenthal [17].

2.4.7 Serum creatinine

Creatinine assay was determined by Jaffe method as described by Wen-Sheng et al. [18].

2.4.8 Serum electrolytes (Sodium, Potassium and Chloride)

Determination of serum electrolytes (sodium, potassium and chloride) was carried out by ion selective electrode method as described by Chacko et al. [19].

2.5 Statistical Analysis

The data obtained were statistically analysed using Analysis of Variance (ANOVA). Bonferroni's test was used for Post-hoc comparisons and $P<0.05$ was considered statistically significant.

3. RESULTS

Administration of the root extract caused significant increase in activities of AST (at higher doses) and ALT (for most doses). While the values for ALP, total protein and albumin were not significantly ($P>0.05$) changed compared with control, the LDH activity increased significantly ($P<0.05$) up to the median dose and reduced significantly ($P<0.05$) for the rest of the doses. At median dose administration, there were significant ($P< 0.05$) changes in the values of all the parameters. The highest administered dose of the extract had no effect on all parameters except a significant diminution of LDH activity (Table 1).

The pod extract, at all administered dose levels, caused significant ($P<0.05$) increase in the activities of AST, ALP and LDH. ALT activity increased significantly at very high dosing of extract. While the level of total protein did not change significantly for all doses of extract, albumin increased significantly ($P<0.05$) for most administered doses and decreased significantly ($P<0.05$) at the highest administered dose (Table 2).

In Table 3, the stem extract showed no significant ($P>0.05$) effect on AST and ALT activities. While the activity of ALP was significantly ($P<0.05$) increased at very high doses of the extract, the LDH activity was significantly ($P<0.05$) increased at all doses of the extract. The stem extract did not affect the concentration of serum total protein, but albumin concentration was significantly ($P<0.05$) reduced at lower doses and significantly increased at the highest dose administered.

Median dose administration of the root extract caused significant ($P<0.05$) increase in the serum concentrations of urea, sodium and potassium. Potassium concentration was also significantly ($P<0.05$) altered by administration of highest doses of extract (Table 4).

At a relatively lower dose, the pod extract caused a significant rise in the level of serum urea. The extract also showed a dose dependent change in the level of creatinine and increased the chloride ion concentration significantly (Table 5).

The stem extract, while having no effect on urea concentration, caused significant ($P<0.05$) increase in the level of creatinine and chloride for all the groups (Table 6). There was no significant effect on serum sodium concentration, but potassium concentration rose significantly ($P<0.05$) when extract was administered at relatively very high doses.

Table 1. Effect of different doses of *Telfairia occidentalis* root extract on serum AST, ALT, ALP, Total Protein, Albumin and LDH of wistar rats (n=4; means±S.E.)

Group	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total protein (g/L)	Albumin (g/L)	LDH (U/L)
1	Distilled Water	69.33±6.5	24.0±0.6	199.7±3.2	63.0±1.7	32.0±2.5	4119.7±61.3
2	Root Extract (250 mg/kgbw)	120.0±7.0	42.3±1.5[a]	189.3±7.4	67.5±4.5	33.5±0.5	4342.7±44.8 [a]
3	Root Extract (750 mg/kgbw)	114.5±22.5	40.5±0.5[a]	141.7±4.4	62.5±0.5	29.0±0.0	4649.5±29.5 [a]
4	Root Extract (1500 mg/kg bw)	148.0±1.0[a]	91.0±2.0[a]	315.0±5.0[a]	47.5±0.5 [a]	22.0±1.0 [a]	4499.0±1.0 [a]
5	Root Extract (2250 mg/kg bw)	143.0±5.0[a]	64.5±2.5[a]	293.3±44.1	68.5±3.5	25.7±0.3	3362.5±37.5 [a]
6	Root Extract (3000 mg/kgbw)	86.3±5.0	22.3±1.9	183.3±3.8	57.5±0.5	26.0±2.0	2975.5±34.5 [a]

[a]*Significant difference (P< 0.05) compared to Group 1.*

Table 2. Effect of different doses of *Telfairia occidentalis* pod extract on serum AST, ALT, ALP, Total Protein, Albumin and LDH of wistar rats (n=4; means±S.E.)

Group	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Protein (g/L)	Albumin (g/L)	LDH (U/L)
1	Distilled Water	82.0±5.0	27.5±0.6	191.3±4.7	66.5±2.8	27.8±2.1	1112.0±28.9
2	Pod Extract (250 mg/kg bw)	118.3±4.9[a]	29.0±1.9	227.3±9.2 [a]	61.8±5.1	35.0±1.2	2464.5±205.2 [a]
3	Pod Extract (750 mg/kg bw)	110.0±1.0[a]	25.7±1.2	286.5±6.5[a]	69.3±4.3	37.3±1.7 [a]	2267.0±226.0 [a]
4	Pod Extract (1500 mg/kg bw)	116.8±2.9[a]	28.5±0.3	282.0±4.8[a]	78.0±3.9	37.3±1.7 [a]	2725.3±156.7 [a]
5	Pod Extract (2250 mg/kg bw)	114.0±0.4[a]	32.8±1.1[a]	281.6±4.4[a]	68.0±1.8	36.5±1.4 [a]	2739.3±203.1 [a]
6	Pod Extract (3000 mg/kg bw)	116.0±0.6[a]	38.0±0.6[a]	288.0±2.0[a]	76.0±3.8	23.0±8.0 [a]	2944.3±183.4 [a]

[a]*Significant difference (P< 0.05) compared to Group 1.*

Table 3. Effect of different doses of *Telfairia occidentalis* stem extract on serum AST, ALT, ALP, Total Protein, Albumin and LDH of wistar rats (n=4; means±S.E.)

Group	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Protein (g/L)	Albumin (g/L)	LDH (U/L)
1	Distilled Water	82.0± 5.0	27.5±0.6	191.3±4.7	66.5±2.8	27.8±2.1	1112.0±28.9
2	Stem Extract (250 mg/kg bw)	94.5±6.9	21.5±1.9	242.7±21.3	60.3±2.9	22.0±2.0 [a]	1895.0±52.9[a]
3	Stem Extract (750 mg/kg bw)	100.3±6.3	21.5±1.3	247.3±8.8	67.3±1.8	22.0±1.9 [a]	1970.0±23.0[a]
4	Stem Extract (1500 mg/kg bw)	107.0±6.9	22.5±5.5	193.5±0.5	59.0±1.0	27.3±1.7	2384.0±65.6 [a]
5	Stem Extract (2250 mg/kg bw)	97.0±2.5	19.7±1.4	312.3±24.7 [a]	60.3±3.1	25.0±2.2	2535.5±122.7[a]
6	Stem Extract (3000 mg/kg bw)	89.0±0.6	26.3±4.5	406.7±41.0[a]	64.3±0.9	31.3±1.3 [a]	2725.0±29.8[a]

[a]*Significant difference (P< 0.05) compared to Group 1.*

Table 4. Effect of different doses of *Telfairia occidentalis* root extract on serum levels of urea, creatinine, sodium, potassium and chloride ions in wistar rats (n=4; means±S.E.)

Group	Treatment	Urea (mmol/L)	Creatinine (µmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
1	Distilled Water	6.0±0.5	77.5±3.2	153.5±1.2	5.4±0.1	163.3±3.1
2	Root Extract (250 mg/kgbw)	3.9±0.2	61.7±1.7	154.7±1.2	5.7±0.1	160.7±4.7
3	Root Extract (750 mg/kgbw)	3.8±0.2	60.0±2.9	148.0±0.6	5.7±0.1	161.7±6.0
4	Root Extract (1500 mg/kg bw)	39.2±1.1[a]	98.5±1.5	163.5±3.5[a]	7.5±0.3[a]	171.5±0.5
5	Root Extract (2250 mg/kg bw)	5.1±0.6	80.0±7.6	152.3±1.2	6.1±0.1[a]	168.0±2.5
6	Root Extract (3000 mg/kgbw)	5.1±0.4	66.7±3.3	150.7±0.9	4.8±0.1 [a]	170.7±1.8

[a]*Significant difference (P< 0.05) compared to Group 1.*

Table 5. Effect of different doses of *Telfairia occidentalis* pod extract on serum levels of urea, creatinine, sodium, potassium and chloride ions in wistar rats (n=4; means±S.E.)

Group	Treatment	Urea (mmol/L)	Creatinine (µmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
1	Distilled Water	4.3±0.1	50.3±1.4	139.0±0.6	4.9±0.1	99.3±2.2
2	Pod Extract (250 mg/kg bw)	8.0±0.4	69.3±2.3 [a]	139.8±0.9	4.7±0.2	100.3±0.3
3	Pod Extract (750 mg/kg bw)	13.4±1.4[a]	74.3±2.2 [a]	139.7±1.2	4.6±0.1	104.7±0.3 [a]
4	Pod Extract (1500 mg/kg bw)	3.9±0.2	49.0±0.6	139.0±0.4	4.9±0.1	104.8±0.6 [a]
5	Pod Extract (2250 mg/kg bw)	3.5±0.5	52.8±3.1	133.7±0.3[a]	5.2±0.3	110.8±0.5 [a]
6	Pod Extract (3000 mg/kg bw)	8.9±2.8	69.0±1.0[a]	143.3±1.8	5.3±0.1	109.0±0.6 [a]

[a]*Significant difference (P< 0.05) compared to Group 1.*

Table 6. Effect of different doses of *Telfairia occidentalis* stem extract on serum levels of urea, creatinine, sodium, potassium and chloride ions in wistar rats (n=4; means±S.E.)

Group	Treatment	Urea (mmol/L)	Creatinine (µmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
1	Distilled water	4.3±0.1	50.3±1.4	139.0±0.6	4.9±0.1	99.3±2.2
2	Stem extract (250 mg/kg bw)	3.8±0.5	69.3±1.7 [a]	137.8±0.8	5.2±0.0	272.0±26.0[a]
3	Stem extract (750 mg/kg bw)	3.0±0.1	79.3±1.8 [a]	139.3±0.5	5.1±0.1	290.5±13.5[a]
4	Stem extract (1500 mg/kg bw)	3.0±0.1	69.0±2.0 [a]	137.0±0.4	5.2±0.0	187.0±4.3[a]
5	Stem extract (2250 mg/kg bw)	2.7±0.4	67.8±2.6 [a]	136.8±0.6	6.0±0.2[a]	229.0±8.7[a]
6	Stem extract (3000 mg/kg bw)	4.1±0.6	68.3±2.9 [a]	138.8±0.5	5.3±0.1[a]	205.5±2.1[a]

[a]*Significant difference (P< 0.05) compared to Group 1*

4. DISCUSSION

Increased serum activity of AST and ALT has been attributed to disruption of liver membrane integrity leading to the leakage of these organ-specific enzymes into the serum. Significant increase in both enzymes has been associated with acute and sub acute liver disorders [20]. Increased serum ALP activity was implicated in biliary obstruction [3]. Although present in almost all tissues, LDH is associated with cardiac tissue damage and is released into the serum if cellular injury occurs [20]. Administration of different doses of the root extract may impact on the cell membrane integrity, but not enough to affect the protein metabolic and secretory function of the liver. Also the bile may have been spared at some dose levels. The extract seemed to have its severest impact at half the maximum dose administered with no significant effect at maximum dose.

The pod extract for all doses administered may have caused disruption of membrane integrity of the liver cells. The extract while preserving protein metabolism, caused increased secretion of albumin into the peripheral blood circulation. This effect of pod extract on albumin was reversed on administering the highest dose. Albumin being the most abundant protein is involved in preserving osmotic pressure, transporting nutrients and metabolites and as protein reserve [21].

The stem extracts at all dose levels caused no liver necrotic injury. The raised ALP activity as a result of high dosing of the extract may indicate induction of obstructive biliary obstruction or effect on other extra hepatic sources such as the bone, placenta and kidney [22-24]. The observed hypoalbuminemia at lower doses of stem extract may result from reduced albumin secretion, malabsorption or dehydration [25]. This was reversed by the maximum dose administration. The significantly progressive increase in LDH activity may result from the effect of extract on hepatic or extra hepatic sources.

Nephrotoxic agents usually affect either or both of the kidney functions which include glomerular filtration and tubular reabsorption and secretion [21]. To an extent, the root extract at the median dose impacted retrogressively on the renal function, which was reversed at maximum exposure to the extract. Concentration of urea in the serum is often used in determining the functional state of the kidney. As nitrogenous metabolite of protein and amino acid catabolism, urea is generated in the liver and distributed to both the intracellular and extracellular fluids [26]. It is less than creatinine in reliability with respect to measurement of glomerular filtration rate as its serum level is affected by diffusion, protein intake and metabolism [21].

Increased serum creatinine level indicates significant renal impairment [27]. Increased serum concentration of sodium has been attributed to loss of Na+ from extracellular fluid, increased hormone dependent renal reabsorption [28] or dehydration [21]. Potassium is a better marker of renal failure as decreased filtration and secretion in distal tubule result in increased serum concentration [26]. Renal loss leading to reduction in serum level is associated with diuresis, nephropathy, diarrhoea, drug use (including steroid hormone) and liver disease [21]. Thus, both the pod and stem extracts may cause mild to significant renal impairment.

5. CONCLUSION

So far this study has shown that the effect of aqueous extracts of *T. occidentalis* root, pod and stem on the liver and kidney is not directly proportional to the dosage administered. While the extracts at onetime showed signs of toxicity even at lower dose levels, toxicity disappeared on higher dosing, and vice versa. This may be an indication that while some of the bioactive principles in the extracts are present in low concentration and can attain biological activity only at high dose or longer administration period, others are present in high concentration and so attain their activity threshold even at very low dose level.

Also the bioactive principles may be cumulative; exhibiting some synergistic and /or antagonistic tendencies either which is achievable with varying dose and duration.

CONSENT AND ETHICAL APPROVAL

Author hereby declare that this research, involving animal studies, was carried out after due approval (of research relevance and design, including research ethics in animal handling and patients consent) by the Departmental Board of Postgraduate Studies, Department of Biochemistry, Faculty of Life Sciences, University of Benin, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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