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Original article

Biochemical Evaluation in Wister Rats (*Rattus Novergicus*) Following Chronic Exposure of Methanol Leaf Extract of *Telfairia occidentalis*

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ABSTRACT: Background: *Telfairia occidentalis* family (*Cucurbitaceae*) is a tropical plant with several biological and pharmacological activities. The historical use of *T.occidentalis* leaves for medicinal purposes by

the local population in Nigeria without adequate information on its toxicity most especially on liver function necessitated this study.

Objective: the present study aimed at evaluating the effects of methanol extract from this plant on biochemical parameters in rats.

Methodology: A total of fifteen (15) wister rats were grouped into 3 groups of 5 animals each. Group A rats serve as control group, while group B and C was treated with 300 and 600 mg/kg of *T. occidentalis* respectively, for 30 days.

Results: Acute oral toxicity shows the extract to be relatively safe at a high dose on acute exposure. However, 30 days administration of methanol extract of *T. occidentalis* cause dose dependents and significant ($P>0.05$) increase in serum activities of ALT, ALP and the level of total protein but had no effect ($P>0.05$) on serum AST activities when compared with the control rats. The extract had no significant effects on body weight gain and liver, spleen, small intestine and heart body weight ratios of the rats but cause significant ($P>0.05$) increased in kidney/body weight ratio when compared with the control values

Conclusions: In conclusion, administration of methanol extracts of *T. occidentalis* at the doses investigated has brought about alterations in the serum activities of ALT, ALP and the level of total proteins but had no effects on serum AST. This may be an indication of selective and local systemic toxicity of plant extract

KEYWORDS: Toxicity, *Telfairia occidentalis*, Organs AST, ALT, ALP, total proteins

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INTRODUCTION

An estimated 90% people of the worlds developing countries rely chiefly on medicinal plants to meet up there therapeutics need (WHO, 2002)¹. In Nigeria and other African country, medicinal plants form an

important component of the natural wealth of the countries. Many local inhabitants of African indiscriminately used most of these plants for managing various diseased states without actually

knowing how relief is brought about or its Safety/toxicity risk².

The inadequate quality control, efficacy and safety validation of medicinal plants has raised concerns over the last decades³. Several warnings have been raised regarding the potential adverse effects of medicinal plants on body tissues especially the liver and kidney⁴. Therefore, the continuous evaluation of these botanicals for safety/toxicity using different animal models is widely encouraged, since the responses by these animals to chemical agents could be translated to human respect. During this safety/toxicity study of plants evaluation of biochemical indices in serum and organs of animals have become the most valuable tools for assessing the integrity and functionality of organs as well as risk assessment, pathological condition and general health status of the body⁵.

Telfairia occidentalis family (*Cucurbitaceae*) is a tropical vine grown mainly as leaves, the leaves constitutes an important component of the diet of many people in the West Africa countries⁶. The green leaves of fluted pumpkin are generally called Ugwu. It is well known in Southern Nigeria because of its pleasant taste. The leaves are low in crude fibre, rich source of protein, oil, vitamins and minerals which enhances, nourish, protect and heal the body⁷. Several biological and pharmacological activities of *Telfairia occidentalis* including antiplasmodial, antioxidant, antidiabetic, hepatoprotective, antimicrobial, haematological, testiculoprotective, antiinflammatory, anticancer, anxiolytic, sedative and anticonvulsant properties as been reported in literature⁸. The historical use of *T.occidentalis* leaves for medicinal purposes by the local population in Nigeria without adequate information on its toxicity most especially on organs function necessitated this study. The serum biochemical parameters evaluated in this study are useful indices of liver function. These parameters when adversely altered in serum will impede the integrity of the liver and thus healthy nature of the animals⁹.

MATERIALS AND METHODS

PLANT SAMPLE

Fresh leaves of *Telfairia occidentalis* were obtained from Bosso Market, Minna, Niger State Nigeria. Taxonomic authentications of the plant was carried out at Departments of Biological Science, Federal University of Technology, Minna.

PREPARATION OF THE EXTRACTION

The fresh leaves of *Telfairia occidentalis* was properly rinsed with clean water, slice into pieces and air dried before being pulverized with

electrical grinding machine. The active portion of the plant was extracted with soxhlet apparatus using methanol at ratio 1:3 (Plant:Solvent). After the extraction, the extract was concentrated using rotary evaporator and the concentrated extract was weighed, stored in an airtight container before being refrigerated until required.

EXPERIMENTAL ANIMALS

A total of fifteen (15) white albino rats (*Rattus norvegicus*) of both sexes with average weight of 120±150g were procured from the Small Animal Holding Unit of the Department of Biochemistry, Federal University of Technology Minna. The rats were kept in clean plastic cages and maintained under standard laboratory conditions (temperature: 22±3°C; photoperiod: 12 h natural light and 12 h dark; humidity: 40-45%)¹⁰.The animals were maintained on standard animal feeds (Bendel feeds and flour mills, Edo state, Nigeria) and tap water *ad libitum*. The principles governing the use of laboratory animals as laid out by the Federal university of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (1997), were duly observed¹¹.

ASSAY KIT

The assay kits for AST, ALT, ALP and total proteins were products of Randox Laboratories Ltd., United Kingdom. All other reagents used were of analytical grade and were prepared in distilled water.

EXPERIMENTAL PROTOCOL

Fifteen (15) Swiss Albino rats were grouped into three (3) consisting of five (5) rats each. Group A (control) received orally, 0.5 ml of distilled water (Vehicle for extract administration) for 30 days while Groups B and C were treated like the control except that they received 300 and 600 mg/kg body weight of the extract for 30days. The administration was done using metal oropharyngeal cannula.

COLLECTION OF BLOOD SAMPLE

Prior to termination of the experiment on day 31, the rats were fasted overnight but still had access to clean water *ad libitum*. Blood samples were collected by cardiac puncture under ether anesthesia. The blood was collected in a clean, dry EDTA free sample bottles and was allowed to stand for 10 minutes at the room temperature and then

centrifuged at 1000 rpm for 15 minutes to get the serum for biochemical analyses.

DETERMINATION OF BIOCHEMICAL PARAMETERS

The biochemical analyses were determined for alkaline phosphatase (ALP) based on methods of Tietz, (1995)¹². Aspartate transaminase (AST) and alanine transaminase (ALT) as described by Reitman and Frankel, (1957)¹³. The serum total protein concentration was estimated by biuret method as described by Gornall et al., (1949)¹⁴.

STATISTICAL ANALYSIS

Data were analyzed using statistical package for social science (SPSS) version 16 and presented as means±SEM. Comparisons between different

groups was done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $P < 0.05$ were considered as statistically significant as described by Yalta (2008)¹⁵.

RESULTS

Acute toxicity (LD₅₀)

The LD₅₀ of methanol leaf extract of *Telfairia occidentalis* was found to be greater than 5,000 mg/kg body weight. No death or sign of toxicity were recorded within 24 h after treatment with the extract (Table 1).

Table 1. Acute toxicity profile of methanol leaf extract of *Telfairia occidentalis*.

Dose (mg/kgbw)	No. of Rats	Mortality	Toxicity
10	3	0/3	No sign of toxicity
100	3	0/3	No sign of toxicity
1000	3	0/3	No sign of toxicity
1600	3	0/3	No sign of toxicity
2900	3	0/3	No sign of toxicity
5000	3	0/3	No sign of toxicity

Table 3. Effect of methanol leaf extract of *T. occidentalis* on relative organ weight of albino rats.

Parameters	300mg/kg <i>T. Occidentalis</i>	600mg/kg <i>T. Occidentalis</i>	Control rats
Kidney	0.006±0.001 ^b	0.006±0.001 ^b	0.005±0.001 ^a
Liver	0.024±0.001 ^a	0.021±0.000 ^a	0.024±0.005 ^a
Heart	0.005±0.001 ^a	0.0053±0.001 ^a	0.005±0.001 ^a
Spleen	0.0042±0.002 ^a	0.004±0.002 ^a	0.0042±0.000 ^a
Small intestine	0.002±0.003 ^a	0.0029±0.001 ^a	0.002±0.002 ^a

BIOCHEMICAL PARAMETERS

The effects of administration of aqueous extract of *Telfairia occidentalis* at doses of 300 and 600mg/kg body weight on biochemical parameters of albino rats are shown in fig 1. Thirty (30) days administration of methanol extract of *T.*

occidentalis cause dose dependents and significant ($P < 0.05$) increase in serum activities of ALT, ALP and the level of total protein but had no significant ($P > 0.05$) effect on serum AST activities when compared with the control rats.

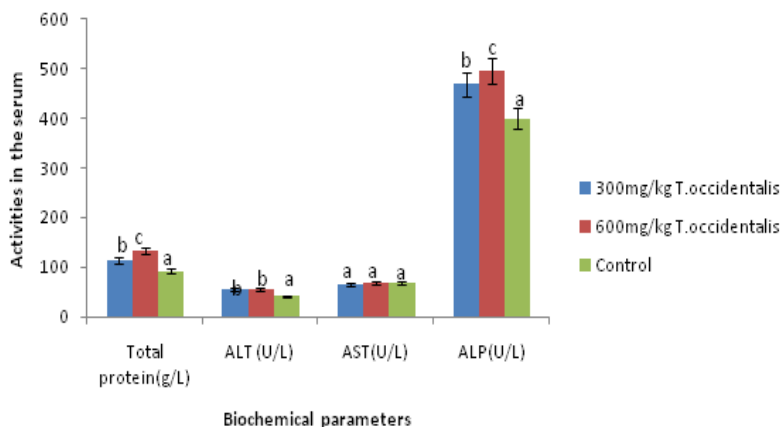


Figure 1. Effect of chronic administration of methanol leaf extract of *Telfairia occidentalis* on biochemical parameters in albino rats.

BODY WEIGHT AND RELATIVE ORGAN WEIGHT

Administration of methanol extract of *Telfairia occidentalis* had no significant ($P>0.05$) effects on body weight gain of albino rats (Table 2). The relative organ weight ratios indicated that the liver, spleen, small intestine and heart body weight

ratios of the rats were not significantly ($P>0.05$) different from those of the control rats (Table 3). However, the extract cause significant ($P<0.05$) increased in kidney/body weight ratio when compared with the control values.

Table 2. Effect of methanol extract of *Telfairia occidentalis* on weight changes of albino rats.

Groups	Initial weight (G)	Final weight (G)	Weight gain(G)
Control rats	183.46±9.51	199.70±8.82	16.24
300mg/kg <i>T. Occidentalis</i>	179.08±5.21	196.48±12.01	17.40
600mg/kg <i>T. Occidentalis</i>	192.97±7.08	209.89±12.01	16.92

DISCUSSION

It is widely accepted that the use of plants-derived principles will offer access to effective medical care for the treatment and managements of diseases through self-medication. However, the major overriding measure as suggested by world health organization in the selection of herbal medicines for health care need is safety (WHO, 2001)¹⁶. In addition to medicinal properties, plants extracts should be safe for consumption without having any adverse effects on the body tissues or organs especially the liver which is the main organ responsible for metabolism of the plant when ingested.

Biochemical parameters such as AST, ALP, ALT and proteins have been accepted and widely use in scientific assessments of liver integrity, since impaired liver activities is always accompanied by alteration in normal activities of these biomarkers⁹. In this study, 30 days administration of methanol extract of *T. occidentalis* at doses of 300 and 600mg/kg body weight cause dose dependents and significant increase in serum activities of ALT, ALP and the level of total protein but had no significant effect on serum AST activities when compared with the control rats.

Alkaline phosphatase has been widely use as biomarker enzyme for assessing the integrity of endoplasmic reticulum and plasma membrane. The significant increase in the activities of this enzyme after 30 days of extract administration suggested that the integrity and functionality of endoplasmic reticulum and plasma membrane has been jeopardised. The extract might have activated *in situ* activities of the enzyme molecule⁵, or increase rate of the enzyme synthesis. This will consequently, effect the rate of ion transport across the membranes of the tissues¹⁷.

The activities of AST and ALT (Transaminase enzymes) can also provide useful information regarding the hepatocellular integrity, since they are biomarkers of compromised liver activities¹⁸, with ALT activities being more valuable than AST¹⁹.

The significant increase in the activities of this enzyme after 30 days of extract administration suggested leakages of this enzyme into the serum. This may results from hepatic metabolism of the extract and hepatic tissue turnover, in response by the body system towards overcoming stress induced by the constituents of the plant extract²⁰. However, the non significant effects of the extract on serum AST activities suggested local systemic and selective toxicity of the plant extract.

The level of total protein can be use as a good indicator of secretory, synthetic and excretory roles of the kidney and liver and kidney²¹. The increase in the level of serum total proteins following 30 days administration of *Telfairia occidentalis* suggests an impaired synthetic integrity of the liver. The extract might have altered the equilibrium in the rate of synthesis and destruction, removal or clearance of total protein, Such increase in total protein could, however, lead to dehydration which is detrimental to cellular homeostasis with consequents effects on the metabolic activities of the liver and consequently the health of the animals²¹.

Organ-body weight ratio is a useful marker of cellular swelling, atrophy or hypertrophy²². Therefore, the absence of an effect on the computed organ-body weight ratios suggest that the extract did not cause any form of swelling, atrophy and hypertrophy on the organs. However, the significant increase in kidney/body weight ratio of rats following 30 days extract administration may be attributed to tissue necrosis since the organ is concerned with the excretion of foreign substances²³.

CONCLUSION

In conclusion, administration of methanol extract of *T. occidentalis* at the doses investigated has brought about alterations in the serum activities of ALT, ALP and the level of total proteins but had no effects on serum AST. This may be an

indication of selective and local systemic toxicity of plant extract.

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