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Effects of Methanol Extract of *Telfairia occidentalis* Seed on Serum Lipid Profile, Biochemical and Antioxidant Activity in Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOOD designed the study, carried out the literature searches and wrote the first draft of the manuscript. Author WAO supervised the experiment, did the statistical analysis and corrected the first draft of the manuscript. Author GO carried out the experiment. All authors read and approved the final manuscript

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ABSTRACT

Aim: This study was designed to investigate the effects of oral administration of methanol extract of *Telfairia occidentalis* seed (METOS) on serum lipid profile, liver biochemical and antioxidant enzymes and lipid peroxidation.
Methodology: Twenty female Wistar rats (165-200 g) were randomly grouped evenly into four and treated as follows; Group A (control), Group B, C and D were administered orally with 20, 40 and 80 mg METOS/kg bw respectively for thirty days. Serum lipid profile, liver biochemical, and antioxidant enzymes activities and lipid peroxidation were analyzed.
Results: Serum cholesterol, low density lipoprotein and aspartate aminotransferase were reduced significantly in animals administered with 20, 40 and 80 mg METOS/kg bw compared with the control (P = 0.05), whereas, 40 and 80 mg METOS/ kg bw significantly increased serum triglyceride (mg/dl) (659.50±53.34, 652.63±29.13) and high density lipoprotein (mg/dl) (101.9±10.70, 112.00±6.40) relative to the control group (368.20±41.89), (63.3±2.82) (P = 0.05). Alanine

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aminotransferase (IU/L) reduced significantly in 40 and 80 mg METOS/kg bw (4.10±0.37, 3.63±0.33) compared with the control (6.60±0.80), while significant reduction in alkaline phosphatase (IU/L) was observed in 20 and 80 mg METOS/kg bw treated groups (3.60±0.82, 3.27±0.4) compared with the control (5.72±0.78) (P = 0.05). Catalase activity level (µ/mg tissue) was statistically increased in 20 and 80 mg METOS/kg bw groups (13.13±1.21, 9.64±1.75) compared with the control (4.56±1.57) and malondialdehyde level (nM/mg tissue) increased significantly in all METOS treated groups (0.05±0.001, 0.04±0.003, 0.08±0.002) compared with the control group (0.02±0.002) (P = 0.05).

Conclusion: In conclusion, the results of this study suggested that METOS has hypolipidemic effect which may further reduce the risk of cardiovascular diseases, it also has hepatoprotective effect and antioxidant activities which ameliorate its lipid peroxidation effect.

Keywords: Telfairia occidentalis; lipid profile; biochemical enzyme; antioxidant; wistar rats.

1. INTRODUCTION

Hyperlipidaemia is a heterogenous group of disorders characterized by high level of lipids in the bloodstream. It may be caused by disorders of some endocrine glands, kidneys, effects of certain drugs, dietary intake containing high amount of fat, risky life style and ageing [1]. It is one of the risk factors in development of atherosclerosis [2].

In Africa traditional medicine, certain oil seeds are employed in the treatment of hyperlipidaemia. *Telfairia occidentalis* is named among Nigerian seed plants that are under investigation for anti-hyperlipidaemic activity [3]. *Telfairia occidentalis* is a tropical vine grown in West Africa as a leaf vegetable and belongs to the family of curcubitaceae [4,5]. Its seeds are usually eaten as snacks, used as soup condiment and as herbal remedy for low sperm count [6,7]. It has been reported to be highly nutritious and contain oil with high amount of polyunsaturated fatty acid and lower amount of saturated fatty acid [8].

Studies have shown that ethanolic extract of *Telfairia occidentalis* seed caused an increase in serum level of cholesterol but decreased serum level of high density lipoprotein [9]. The leaf of *Telfairia occidentalis* supplemented in diet has however been reported to lower plasma cholesterol level, low density lipoprotein level and lipid peroxidation level in rats fed with cholesterol-rich diet [3]. The reliability on the curative or preventive effects on this seed must also consider its safety, for example, its effects on the liver, being the organ that metabolizes ingested substances can be determined by measuring the level of transferases which are indicators of drug induced liver toxicity [10]. Result from an *in vitro* study also suggested that the leaf has free radical scavenging activity [11]. Several studies have investigated supplement diets effects of the *Telfairia occidentalis* leaf and seed on lipid profile and liver biochemical enzymes at different dosages [3,12]. This study is therefore aimed at investigating the effects of methanol extract of *Telfairia occidentalis* seed (METOS) on serum lipid profile, biochemical and antioxidant activity in female Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant

2.1.1 Collection and identification of plant

Fluted Gourds of *Telfairia occidentalis* were purchased from Orie Ugba market in Umuahia, Abia State. The whole plant was authenticated at the herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The sample of the plant was assigned with the voucher number: 108846.

2.1.2 Method of extraction

The seeds obtained from *Telfairia occidentalis* gourd were decocted and air dried until a consistent weight (1,678 g) was obtained at about the fourth week. The seeds were blended into powdery form, which was soaked in six liters of methanol for 48 hours, during which period it was stirred at intervals and filtered at the end of the duration. The filtrate was concentrated by freeze drying. Methanol extract of *Telfairia occidentalis* (METOS) seed had a percentage yield of 4.73 and appeared brownish, sticky and oily. It was then stored at room temperature throughout the experiment.

2.2 Experimental Animals

Twenty (20) female Wistar rats weighing between 165-200 g were obtained from Igbinedion University animal house. The animals

were kept in well ventilated plastic cages and maintained under standard laboratory conditions (12 hours natural light and 12 hours dark cycle). They were fed with pelletized feed and allowed free access to water. The animals were acclimatized for two weeks before commencement of extract administration. All the animal care and experiment were carried out in accordance to NIH (No. 85-23, revised 1985).

2.3 Experimental Design

The animals were randomly distributed into four groups (n = 5). The extract was orally administered for a period of 30 days as follows;

Group A (control) was administered distilled water;
Group B was treated with 20 mg METOS/kg bw;
Group C was treated with 40 mg METOS/kg bw
Group D animals were administered with 80 mg METOS/kg bw.

2.4 Tissue Collection

The animals were sacrificed after the last day of administration (day 31) by cervical dislocation and dissected through the linea alba. Blood sample was collected by cardiac puncture and serum obtained from the blood was used for estimation of cholesterol, triglyceride, HDL and LDL. About 5 g of liver was harvested, homogenized in phosphate buffer saline and supernatant obtained was used for estimation of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels. A portion of the liver was also fixed in 10% formalin for histology.

2.5 Lipid Profile

Serum cholesterol, triglyceride and high density lipoprotein levels were determined using Randox kits manufactured by Randox Laboratories, UK. Cholesterol was assayed according to the method of Allain et al. [13]. These biochemical parameters were measured using spectrophotometry method.

2.6 Liver Enzymes

Alanine Amino-Transferase (ALT) and Aspartate Amino-Transferase (AST) were measured using the modified method of Reitman et al. [14]. 0.1 ml of liver supernatant was mixed with 0.5 ml of phosphate buffer (L-alanine) and (L-aspartate) for ALT and AST respectively. Incubated the mixture for 30 minutes at 37℃ and then 0.5 ml of 2, 4-dinitrophenylhydrazine was mixed with the mixture and allowed to stand for 20 minutes at room temperature, 5 ml of 0.4 mol/l sodium hydroxide was added and the absorbance of the solution was read after 5 minutes at a wavelength of 546 nm.

Alkaline phosphatase (ALP) level was determined using Randox kits manufactured by Randox Laboratories, UK. The procedure was carried out as described in the kit's manual.

2.7 Histology of the Tissues

Liver was harvested and immediately fixed in 10% formalin for at least 5 hrs. Each sample was dehydrated using ascending grades of alcohol. It was cleared with two changes of xylene, embedded in paraffin wax, trimmed, nicked and sectioned using a microtome and stained using haematoxylin and eosin (H&E) for the purpose of determining the general morphology.

2.8 Antioxidant Activity

Superoxide dismutase (SOD) level was estimated according to the method of Misra and Fridovich, [15] while catalase level was determined according to the method of Aebi, [16].

2.8.1 Thiobabituric acid (TBARS) assay

This was done according to the method of Rice-Evans et al. [17].

2.9 Statistical Analysis

Data from each group were expressed as mean ± standard error of mean (Mean ± SEM). The data were analyzed with one way analysis of variance (ANOVA) followed by Waller-Duncan's post hoc test. *P*–equals to 0.05 was considered significant. The statistical packages used were Microsoft excel and Statistical Package for Social and Sciences (SPSS), version 20.

3. RESULTS

3.1 Effects of Methanol Extract of *Telfairia occidentalis* Seed (METOS) on Lipid Profile

Table 1 shows significant decrease in serum cholesterol level in all the experimental groups

when compared with the control group, also, serum triglyceride level increased significantly in groups treated with 40 mg and 80 mg METOS /kg bw relative to control group (P =0.05). There was also an increase in high-density lipoprotein level in the groups treated with 40 and 80 mg METOS /kg bw when compared with the control group while serum low-density lipoprotein level decreased in all the experimental groups when compared with the control group (P = 0.05).

3.2 Effects of Methanol Extract of *Telfairia occidentalis* seed (METOS) on Biochemical Enzymes in the Liver

Fig. 1 shows that there was a reduction in AST level in all the experimental groups when compared with the control group (P = 0.05). There was also a reduction in the ALT level in the groups treated with 40 and 80 mg METOS /kg bw when compared with the control group (P = 0.05). Liver ALP level was significantly reduced in the groups treated with 20 mg METOS /kg bw and 80 mg METOS /kg bw when compared with the control group (P = 0.05).

3.3 Effects of Methanol Extract of *Telfairia occidentalis* seed (METOS) on Liver Histology

The morphology of hepatocytes in the control group and in all the experimental groups appeared normal.

3.4 Effects of Methanol Extract of *Telfairia occidentalis* Seed on Liver SOD, CAT and MDA Level

Table 2 Table 2 shows antioxidant enzymes and MDA activities in all the experimental animals. There was a significant increase in the catalase level in the groups that received 20 and 80 mg METOS/kg bw when compared with the control group (P = 0.05). There was no significant difference in SOD level in all the test groups when compared with the control group. There was a significant increase in MDA level in all the experimental groups when compared with the control group (P = 0.05).

Table 1. Effect of methanol extract of *Telfairia occidentalis* seed (METOS) on cholesterol, triglyceride high-density lipoprotein and low-density lipoprotein

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	High-density lipoprotein (mg/dl)	Low-density lipoprotein (mg/dl)
Control	758.00±28.50	368.20±41.89	598.65±33.04	598.65±33.04
20 mg METOS/kg bw	415.00±61.99*	294.18±46.85	60.58±5.67	492.74±44.47*
40 mg METOS/kg bw	326.05±49.75*	659.50±53.34*	101.90±40.71*	125.07±13.70*
80 mg METO /kg bw	348.80±29.13*	652.63±169.09*	112.00±9.40*	102.55±19.70*

*Values are expressed as Mean ± SEM (n=5), *P = 0.05 was considered significant compared to control group*

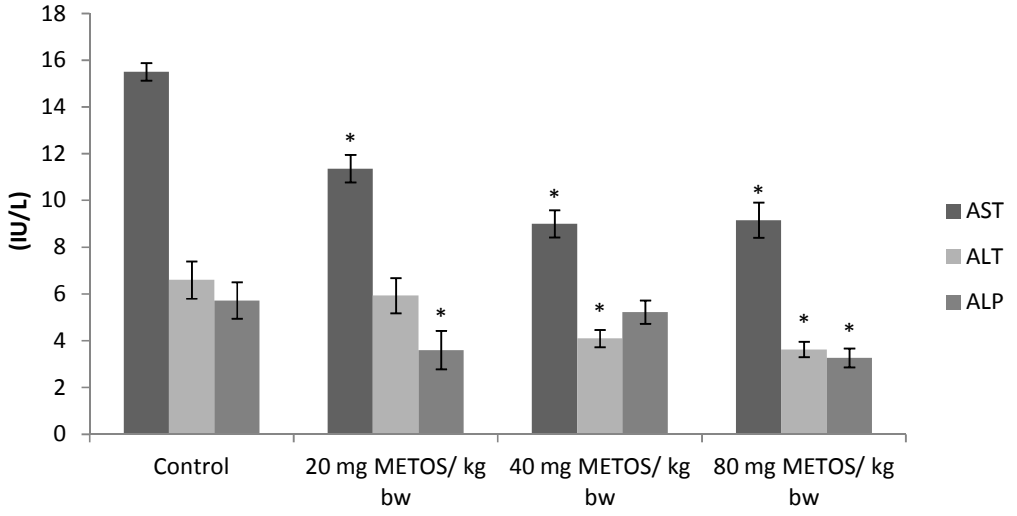

Fig. 1. Effects of METOS on liver AST, ALT and ALP level in female Wistar rats

*Bars are expressed as Mean ± SEM (n=5), *P = 0.05 was considered significant compared to control group*

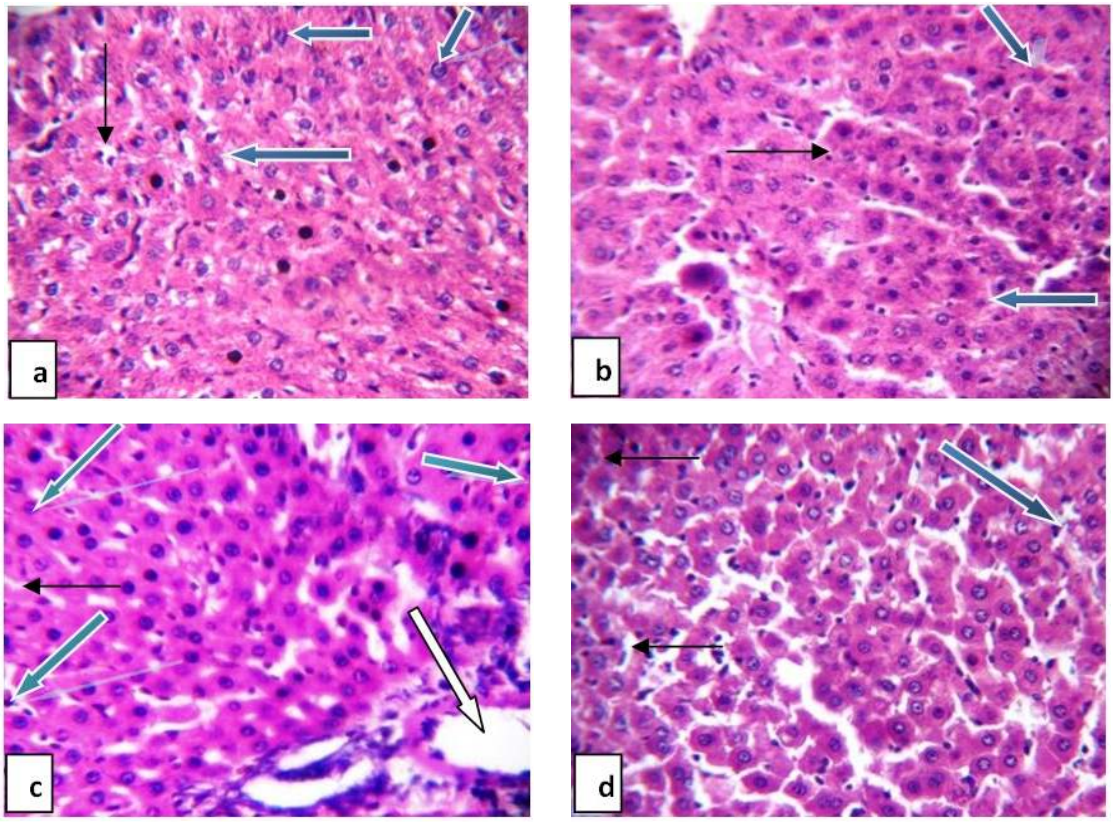

Fig. 2. Photomicrograph of a liver section stained by haematoxylin and eosin (a) control (b) 20 mg METOS/ kg bw (c) 40 mg METOS/kg bw (d) 80 mg METOS/kg bw

(a) shows the sinusoids appear very mildly infiltrated by inflammatory cells (slender arrow). The hepatocytes show several normal morphology (blue arrow) (b) shows the sinusoids appear normal (slender arrow) without infiltration of inflammatory cells. Several other hepatocytes show normal morphology (blue arrow). (c) shows the sinusoids appear normal (slender arrow) without infiltration of inflammatory cells. The hepatocytes show normal morphology (blue arrow). No pathological lesion seen. (d) shows the sinusoids show very mild infiltration of inflammatory cells (slender arrow). The hepatocytes show normal morphology (blue arrow)

Table 2. Effects of methanol extract of *Telfairia occidentalis* on liver SOD, CAT and MDA level

Group	SOD (U/mg)	CAT (µ/mg tissue)	MDA (nM/mg tissue)
Control	67.20±12.22	4.56±1.57	0.01±0.002
20 mg METOS /kg bw	85.00±5.00	13.13±1.21*	0.05±0.001*
40 mg METOS /kg bw	73.80±8.78	5.28±3.36	0.04±0.003*
80 mg METOS /kg bw	78.33±22.29	9.64±1.75*	0.08±0.002*

*Values are expressed as Mean ± SEM (n=5), *P = 0.05 was considered significant compared to control group*

4. DISCUSSION

This present study shows that methanol extract of *Telfairia occidentalis* seed reduced serum cholesterol level, the observed decrease was more pronounced in animals given 40 mg METOS/kg bw. This result was in agreement with the report of Onuegbu et al. [12] and Adaramoye et al. [3] in which supplement diets containing *Telfairia occidentalis* seeds and leaves were used. Although, Eseyin et al. [10] reported that ethanol seed extract of *Telfairia occidentalis* increased serum cholesterol level. The observed decrease in cholesterol level in this study may be as a result of high amount of polyunsaturated fatty acid present in *Telfairia occidentalis* seed which is well known to possess cholesterol lowering effect [18].

The increase observed in the serum high density lipoprotein level in this study was dose dependent. Onuegbu et al. [12] previously

reported that *Telfairia occidentalis* seed incorporated in animal feed caused increased serum high density lipoprotein level. The increase in HDL in this study might be responsible for lowering effect of *Telfairia occidentalis* seed on serum cholesterol level observed in this study. HDL play a protective role against cardiovascular diseases [19] majorly by promoting reverse transport of cholesterol by scavenging excess cholesterol from peripheral tissues and subsequently causing the esterification of the cholesterol using lecithin cholesterol acyltransferase and delivering them to the liver and other steroidogenic organs for bile synthesis and eventual excretion from the body [20,21].

In this present study, the increase in triglyceride level observed in 40 and 80 mg METOS/kg bw contradicts the findings of Ashkok et al. [22] in which the triglyceride level decreased. This increase may be caused by the presence of high content of unsaturated fatty acids like oleic acid in *Telfairia occidentalis* seed which is also found in triglycerides [4,23,24]. The observed increase in triglyceride level in this study may help in regulation of energy balance in the body, although its high amount may be a risk factor for cardiovascular disease [25].

The decrease in the low-density lipoprotein (LDL) level observed in this study is in agreement with the findings of Ugwu et al. [26] and Onuegbu et al. [12] in which the leaf of *Telfairia occidentalis* and a seed supplemented diet caused a reduction in low-density lipoprotein level. This effect could be due to the activity of the fiber content of *Telfairia occidentalis* seed [27]. Fiber is known to decrease LDL-C by interrupting cholesterol and bile acid absorption and increasing LDL receptor activity [28] LDL is known to facilitate transport of cholesterol into cell [29] thus, the observed reduction in LDL justifies the cholesterol lowering effect of *Telfairia occidentalis* observed in this study, thereby, further reducing the risk of cardiovascular disease.

Methanol extract of *Telfairia occidentalis* seed also reduced liver ALP level in this present study, this observation contradict the report of Ejike et al. [30]. This discrepancy may be due to difference in dosages used. The decrease observed in AST, ALT and ALP level in this study suggests that METOS is well tolerated by the liver. The destruction of liver architechture is the principal culprit for the elevation of these liver enzymes. This destruction often occurs in the presence of high amount of toxins and xenobiotics which becomes challenges to the liver [31].

The histological section of the liver in this study showed normal morphology of the sinusoids and hepatocytes, this further buttresses the reduction in the results of AST, ALT and ALP in liver tissue particularly in the groups treated with 20 and 40 mg METOS/kg bw. This therefore depicts that METOS is well tolerated by the liver. Thus, these results suggest that METOS may not pose a threat to the liver architecture.

The increase in catalase level observed in this study is similar to the report of Ajani and Akinyemi, [32] in which both ethanol and aqueous extract of *Telfairia occidentalis* seed caused an increase in catalase level in testosterone induced benign prostate hyperplasia in Wistar rats. The seed have been reported to have high content of vitamin C which may have contributed to its antioxidant capacity. The increase observed in the MDA level in this study is in agreement with Saalu et al. [33] who reported that administration of *Telfairia occidentalis* leaf extract to rat at high dosage caused increased lipid peroxidation. The MDA level observed in this study is however in contrast to the report of Ajani and Akinyemi, [32]. It has been reported that unprocessed *Telfairia occidentalis* seed contains some anti-nutritional factors such as tannin, saponin, oxalates, phytates, trypsin inhibitors and lectins which can be removed by perboiling [34]. The lipid peroxidation level may therefore be reduced should the seed be subjected to heat as observed in a study by Kuku et al. [34]. The increase in catalase level may be able to neutralize any possible effect of METOS.

5. CONCLUSION

In conclusion, the results of this study suggested that METOS has hypolipidemic effect which may further reduce the risk of cardiovascular diseases, it also has hepatoprotective effect and antioxidant activities which ameliorated its lipid peroxidation effect.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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