

Hypolipidemic, Hepato-reno protective and antioxidant effects of *Telfairia Occidentalis* (TO) aqueous leaf extract in Sprague-Dawley rats

^{1*}²Igbayilola Y.D & ¹Ogunkoya O.O

¹Biology Unit, Dept of Science Lab Technology, D.S.Adegbenro ICT Polytechnic, Eruku-Itori, Ewekoro, Nigeria

²Nutrition, Endocrinology and Metabolism Unit, Dept of Physiology, College of Medicine, University of Lagos, Akoka, Nigeria

^{1*}E-mail: princeyai85@gmail.com

Phone: +2348161927278

ABSTRACT

The use of traditional medicine in the treatment and management of diseases in the African continent cannot fade away and this could be attributed to the socio-cultural, socio-economic, lack of basic health care and qualified personnel. The antihyperlipidemic, Hepatoprotective, renoprotective and antioxidant efficacy of *Telfairia Occidentalis* (TO) aqueous leaf extract were studied in male Sprague-Dawley rats twenty-four (n=24). The animals were grouped into four of six rats thus: Group A (control) were given normal saline (10mg/Kg); Groups B, C and D, received, 50mg/kg, 100mg/kg and 150mg/kg *Telfairia Occidentalis* aqueous leaf extract for fourteen (14) days respectively. Blood samples were collected for biochemical analysis. Result from the study showed significant reduction in aspartate amino transferase (AST) and alanine amino transferase (AST) at 100mg/kg when compared with the group A (p<0.05) while ALT was significantly elevated at 50mg/kg with significant elevation in alkaline phosphatase (ALP) at all doses tested when compared with the group A (p<0.05). The lipid profile analysis showed significant elevation in cholesterol (CHOL), high density lipoprotein (HDL) and low density lipoprotein (LDL) levels at all the doses tested (p<0.05) while triglyceride (TG) was significantly reduced at 100mg and 150mg/kg when compared with group A (p<0.05). The protein and albumin level were all elevated but not significant at all the doses tested when compared with group A (p>0.05) while creatinine level was significantly reduced at 50mg/kg when compared with group A (p<0.05). The results from the antioxidant analysis revealed significant elevation in antioxidant enzymes' activities, when compared with the group A (p<0.05), while lipid peroxidation index's malondialdehyde, (MDA) which significantly reduced (p<0.05) in all the doses tested when compared with control group. Results from the current study revealed that TO have anti-hyperlipidemic, Hepatoprotective, renoprotective and antioxidant activities at low dose, however, care has to be taken during therapeutic use as high dose elevated LDL and the activities of liver and kidney enzymes which may be deleterious to the body functions.

Keywords: antioxidant, cholesterol, protein, Sprague-Dawley, *Telfairia Occidentalis*.

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1. INTRODUCTION

The use of plants in traditional medicine referred to as herbalism or botanical medicine (Evans, 2002) falls outside the mainstream of the Western or Orthodox medicine. It has been estimated that about two third of the world's population (mainly in the developing countries) rely on traditional medicine as their primary form of health care (Sumner, 2000). The use of traditional medicine in the treatment and management of diseases in the African continent cannot fade away and this could be attributed to the socio-cultural, socio-economic, lack of basic health care and qualified personnel (Eujoba et al., 2000). Plants contain active components such as flavonoids, glycosides, saponins, tannins, etc., which possess medicinal properties that are harnessed for the treatment of different diseases (Chevalier, 2000). The active ingredients for a vast number of pharmaceutically derived medications contain components originating from phytochemicals. These active substances that contain the healing property are known as the active principles and are found to differ from plant to plant. *Telfairia occidentalis* is an edible vegetable plant that belongs to the family Cucurbitaceae. It is a tropical vine grown mainly in West Africa for its vegetable (Akoroda, 1990). In Nigeria, it is known locally as Ubong by Ibibios, Ugu by Igbos and Iroko by Yorubas. The Ghanians refer to it as okrobonka while to the Sierra- Leoneans, it is known as Oroko (Abiose, 1990).

The concentration of photosynthesis in leaves makes them rich in protein, minerals and sugar. Be-cause of their nutritional value leaves are prominent in the diet of many animals, including humans as leaf vegetables (Leaf, 2011). The leaf has high nutritional, medicinal and industrial values being rich in protein 29%, fat 18%, minerals and vitamins 20% (Ndor, E., Dauda S. N., and Garba, M. N., 2013). The aqueous extract of *T. occidentalis* has been shown to be hepatoprotective against garlic-induced oxidative stress (Olorunfemi et al., 2005, Oboh et al., 2007), while both aqueous and ethanolic extracts have demonstrated hypoglycaemic properties both in normoglycaemic and alloxan-induced diabetic rats (Zhang and Yao, 2002). Studies have also shown the haematinic capacity of this plant hence the use of the concoction of fresh leaves as a high-value health tonic for impotent men and a cheap and fast remedy for acute anemia (Kayode and Kayode, 2011 Nwozo et al., 2004). It was revealed according to Veral et al., (2014) that the nutritional potentials of fluted pumpkin seed shell served majorly as a source of dietary fibre. Also most cultures consider fruit shells as waste in the strictest sense and therefore avoid their use even in earthnomedicine (Verla et al., 2012).

The seeds are cooked and eaten like beans and the shoots and leaves are eaten like vegetables. The leaves contain vitamins and minerals the body needs to stay healthy. The leaves are also a good source of iron (*Telfairia occidentalis*, 2009). *Telfairia occidentalis* is a very good vegetable plant, popularly known in Nigeria for its ornamental purposes. It is hard to believe that anyone would ever think of consuming its leaves as a meal. Although, there are claims concerning the medicinal/health benefits of the leaves which the consumers are not even aware of due to lack of information. Moreover, *Telfairia occidentalis* is going to extinct in most countries especially in Nigeria, where limited research work has been done to verify the claims concerning the nutritional-health values, however the antilipidemic and antioxidant effects of *Telfairia occidentalis* in male Sprague-Dawley rats is largely unknown.

2. MATERIALS AND METHOD

Plant collection and extraction

Telfairia occidentalis leaves were gotten from Itori Ewekoro Local Government Area of Ogun State, Nigeria and the collected samples were identified at FRIN Ibadan Oyo by Mr. Esimekhuai D.P.O. with a voucher number of 110609. The fresh leaves of *Telfairia occidentalis* were oven dried and the required weight was obtained. The dried leaves were powdered using mortar and pestle. 250g of powdered leaves were macerated using distilled water for 48 hours. The extract was filtered and evaporated at 40°C under reduced pressure. The yield of the dark browned colored dried extract obtained was 38.5g and the weighed extract was stored at 4°C until use in which case distilled water was used for reconstitution immediately and was given orally to the experimental animals

Experimental animals

Twenty four (N=24) female Sprague- Dawley rats weighing between 150-200g were obtained from the animal house of the College of Medicine of the University of Lagos. They were kept in well-ventilated, hygienic compartments maintained under standard environmental conditions, acclimatized for three weeks before the experiment. They were fed with standard rodent diet and water ad libitum. The experimental procedures used were in accordance with the provisions of the Experimentation Ethics Committee on Animals Use of the College of Medicine of the University of Lagos, Lagos State and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals.

The extract was given orally to the rats for two weeks.

Group A- Control (10mg/kg of normal saline).

Group B- 50mg/kg of the extract.

Group C- 100mg/kg of the extract

Group D- 150mg/kg of the extract.

Blood sample collection

Blood collection was done at the Research Laboratory of the Physiology Department of the University of Lagos. Blood Samples were collected from each rat for biochemical and antioxidant analysis. The capillary tube was inserted into the medial canthus of the eye (30 degree angle to the nose) A slight thumb pressure was enough to puncture the tissue and enter the plexus/sinus. In order to get serum, blood collected in the eppendorf tube was allowed to clot for about 5 minutes and then centrifuge at 3,000 rpm for 10 minutes. The serum was separated into another eppendorf tube and labelled accordingly.

Biochemical analysis

Serum high density lipoprotein (HDL) was determined using Randox laboratories (England) kit. The serum total cholesterol was determined using Randox laboratories (England) kit based on enzymatic end point method. Serum triglyceride was determined using Hi-tech diagnostic kit. The low density lipoprotein level was calculated from the value of serum HDL, cholesterol and triglyceride level.

Antioxidant analysis

To examine how the different conditions affect the antioxidants concentration and lipid peroxidation in the animals, the levels of superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) and malondialdehyde (MDA) were assessed. Oxidative analyses of the plasma were carried out using previously described standard methods (Morakinyo et al, 2011). Briefly, the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems, malondialdehyde (MDA) was estimated with the method of Uchiyama and Mihara (Uchiyama et al, 1978). The reduced glutathione (GSH) content of the liver homogenate was determined using the method described by Van Dooran et al. (1978) while the activity of the SOD enzyme was determined according to the method described by Sun and Zigman (Sun and Zigman, 1978). Catalase (CAT) activity was determined by measuring the exponential disappearance of H₂O₂ at 240nm and expressed in units/mg of protein as described by Aebi (Aebi, 1984).

Phytochemical screening of of *Telfairia occidentalis*

The phytochemical screening of the plant was carried out on dried sample as described by Harbone, (1973) to identify the active components present in *Telfairia occidentalis*

Acute Toxicity test (LD₅₀) of *Telfairia occidentalis*

The acute toxicity test was carried out by as described by Lorke (1983).

Statistical Analysis

The collected data were analyzed and expressed as mean +/- SEM using SPSS. Unpaired t-test was used. The significant level taken was P<0.05.

3. RESULTS

Phytochemical Analysis

Phytochemical screening result showed the presence of the following phytochemicals;

- Molisch ++
- Fehlings++
- Flavonoid ++
- Magnesium Chip++
- Terpenoids +
- Glycosides ++
- Tannins +
- Saponins ++

Table I: Stage I of the acute toxicity (LD₅₀) of the *Telfairia occidentalis* aqueous leaf extract

GROUPS	DOSAGE (mg/kg)	MORTALITY
Group B	10	Nil
Group C	100	Nil
Group D	1000	Nil

Table II: Stage II of the acute toxicity (LD50) of the *Telfairia occidentalis* aqueous leaf extract

GROUPS	DOSAGE (mg/kg)	MORTALITY
Group B	1600	Nil
Group C	2900	Nil
Group D	5000	ST

The AST level at 100ml/kg and 150mg/kg was significantly reduced ($p < 0.05$) when compared with group A. The ALT at 50ml/kg was significantly elevated ($p < 0.05$) when compared with group A. The ALP level was significantly elevated ($p < 0.05$) in all the tested doses when compared with group A. The CHOL levels at 50ml/kg, 100ml/kg and 150ml/kg were significantly elevated when compared with group A $p > 0.05$. The TG levels at 100ml/kg and 150ml/kg were significantly reduced ($p < 0.05$) when compared with group A. The HDL and LDL levels at all the tested doses were significantly increased ($p < 0.05$) when compared with group A. The results are illustrated in table III.

The protein and albumin levels at all the tested doses was significantly increased ($p < 0.05$) when compared with group A with significant reduction ($p < 0.05$) in creatinine levels in all the tested doses. The results are illustrated in table III. The SOD activities in all the groups tested were significantly elevated ($p < 0.05$) when compared with group A. The CAT activity at 50ml/kg was significantly decreased when compared with group A and the MDA activity was significantly elevated ($p < 0.05$) in group 50mg/kg and significantly reduced ($p < 0.05$) at 100mg/kg and 150mg/kg when compared with group A. The results are illustrated in table IV.

Table III: *Telfairia occidentalis* aqueous leaf extract efficacy on lipid profile, liver and kidney function tests

PARAMETERS	A	B	C	D
AST u/l	58.50±4.38	57.40±7.51	35.50±3.12*#	39.25±6.61*#
ALT u/l	17.67±0.67	25.20±2.65*	20.00±1.29	17.00±4.24#
ALP u/l	68.00±3.98	121.60±4.86*	157.25±16.15*#	111.00±12.43*α
Cholmmol/l	2.27±0.13	2.72±0.19	2.53±0.21	2.55±0.16
TG mmol/l	1.18±0.07	1.14±0.13	0.95±0.16*	0.70±0.11*#
HDL mmol/l	1.25±0.10	1.56±0.13*	1.40±0.20	1.40±0.16
LDL mmol/l	0.48±0.10	0.62±0.11	0.70±0.07*	0.83±0.06*#
Protein g/l	48.67±3.73	51.60±1.81	47.50±3.66	50.25±3.20
Albumin g/l	21.33±0.49	24.40±1.86	22.25±1.03	24.50±2.10
Creatinine μmol/l	34.30±1.96	27.92±0.36*	29.95±1.98	32.38±1.79

Shows a significant interaction at $p < 0.05$ for ALT, ALP, CHOL, TG, HDL, LDL, PROTEIN, ALBUMIN and, using student t-test. Group A: Normal saline (1ml/100 g of rat), B: (50mg/1kg of rat) and C: (100 mg/1kg of rat). Group D (150 mg/1kg of rats).

Table IV: *Telfairia occidentalis* aqueous leaf extract efficacy on antioxidant enzymes

PARAMETERS	A	B	C	D
SOD (mg/ml)	1.11±0.03	1.48±0.02*	1.47±0.02*	1.43±0.02#
CAT (mg/ml)	7.25±2.35	10.10±0.23	9.49±0.09	8.32±1.01#α
GSH (µmol/ml)	599.62±24.38	835.76±19.26*	784.90±7.51*#	647.36±8.26*#α
MDA (nmol/ml)	8.74±0.07	8.53±0.08*	8.71±0.04#	8.01±0.05*#α

*Shows a significant interaction at $p < 0.05$ for GSH, SOD, CAT, and MDA, using student t-test. Group A: Normal saline (1ml/100 g of rat), B: (50mg/1kg of rat) and C: (100 mg/1kg of rat). Group D (150 mg/1kg of rats).

4. DISCUSSION

Telfairia occidentalis has been utilized in many industrial processes; it also provides an appreciable source of income for small farm families. Results from the present study showed increase in liver enzyme activities at 150mg/kg; this showed that there was liver damage while at doses 50mg/kg and 100mg/kg, the enzymes' activities were not significantly elevated and so there is no problem with liver function.

Moreover, the kidney enzymes activity were not significantly elevated as well at dose 50mg/kg and 100mg/kg and so aqueous leaf extract of TO is safe at these doses. In addition, there was elevation in cholesterol and low density lipoprotein levels at 150mg/kg while the HDL which is regarded as good cholesterol was of the increase including TG which showed significant decrease in all the tested doses.

TO may be effective at 50-100mg/kg doses. There have been reports on the lipid profile of various plants and some of which is in accordance with the present study (Ikeda and Sugano, 1998; Harword et al., 2005; Venkatesan et al., 2003; Vinson et al., 1998). Thus, there could be alterations in the concentration of the various lipid metabolism and predisposition of the heart to atherosclerosis and its associated coronary heart diseases.

Therefore, results from lipid profile showed that at minimal dose it possesses anti-hyperlipidemic effect, though literature has shown that it is used as anti-hypercholesterolemic in ethnobotany (Nwozo *et al.*, 2004). The SOD and GSH properties were investigated because of their ability to work hand in hand, SOD catalyses the breakdown of superoxide, the most common free radical in the body into oxygen and hydrogen peroxide while GSH catalyses the breakdown of hydrogen peroxide to water.

Furthermore, there was reduced production of the oxidative radicals from the result of the antioxidant assay and this possibly could prevent disease in the body, this is in agreement with previous study which reported that the presence of antioxidant and antimicrobial properties, its minerals (especially Iron), vitamins (especially vitamin A and C) and high protein contents (Kayode and Kayode, 2011). This could be due to the presence of secondary metabolites like tannins, glycosides, saponins, fehlings and terpenoids. There have been reports on antioxidant activities of various plants, some of which correlates with the present study.



For instance, *Pelargonium reniforme* which is used locally for liver disorders, has strong antioxidant activities as a result of its tannin and flavonoid content (Fernandes et al., 2004). *Mallotus oppositifolium*, a Nigerian plant rich in flavonoids has been said to possess antioxidant as well as anti-inflammatory activities (Farombi et al., 2001). The increase in antioxidant enzymes' activity observed showed the ability to fight diseases in the body. In conclusion, results of the present study revealed that TO possesses anti-hyperlipidemic and antioxidant activities at minimal dose, this is in agreement with its use in folk medicine in combating many diseases in the body, however, since it is dose dependent care has to be taken during its use as it has potential to increase LDL and the activity of liver and kidney enzymes.

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