BIOCHEMICAL AND ANTIOXIDANT EFFECTS OF *TALINUM TRIANGULARE (WATER LEAF)* IN FEMALE SPRAGUE -DAWLEY RATS

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Abstract : Therapeutic properties of medicinal plants are very useful in the treatment and healing various diseases and the advantaged of these plants is been 100% natural with little or no side effects. The present study seeks to investigate the biochemical and antioxidant effects of aqueous leaf extract of Talinum triangulare (water leaf) in female Sprague -Dawley rats. Twenty Four (24) female Sprague -Dawley rats weighing 150-180gram were grouped into 4 of 6 rats each. Group A: control; administered (10ml/kg body weight) of normal saline. The other groups were administered the aqueous leaf extract of Talinum triangulare thus: group B -50mg/kg, group C - 100mg/kg, and group D - 150mg/kg. After fourteen (14) days, blood samples were collected for lipid and antioxidant analysis. The results revealed significant increase in AST (aspartate amino transferase) at 150ml/kg, when compared with the group A S(P>0.05). The ALT (alanine amino transferase) was significantly decreased (P>0.05) at 100ml/kg when compared with group A and group B with significant elevation at 150ml/kg. The ALP (alkaline phosphatase) was significantly increased in all the tested doses (P>.0.05). Results from the lipid profile analysis showed significant decrease in all the doses tested (P>0.05) for cholesterol (CHOL), triglyceride (TG) and high density lipoprotein (HDL) levels except for CHOL and HDL which showed significant increase (P>0.05) at 150ml/kg. The low density lipoprotein (LDL), protein, and albumin levels showed significant increase in the tested doses expect, protein and albumin which was significantly decreased (P>0.05) at 100ml/kg and 150ml/kg. Also the creatinine level was significantly decreased in all the doses tested (P>0.05). The antioxidant assay revealed significant increase in glutathione, GSH, superoxide dismutase, SOD and Catalase, CAT (P>.05) at all doses, expect SOD which was significantly decreased at 150mg/kg. The malondialdehyde, MDA was significantly decreased (P>0.05) in all the tested doses. Our findings showed that aqueous leaf extract of Talinum triangulare administration at 50mg/kg, 100mg/kg and 150mg/kg have no adverse effect on renal functions. However, at 150ml/kg the aqueous leaf extract of Talinum triangulare gave rise to increase in liver enzymes' activity, increased low density lipoprotein as well as oxidative radical productions. Finally excessive intake should be avoided since it is dose dependent because of its ability to increase risk of obesity, cardiovascular disorder and liver damage.

Keywords: Talinum triangulare, Sprague- Dawley, antioxidant, cholesterol, albumin.

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Introduction

The utilization of plant based product in food supplements and health industries increased tremendously for the past decades globally, this is believed to be due to carcinogenic related problems associated with the usage of artificial or chemical products. Therapeutic properties of medicinal plants are very useful in the treatment and healing various diseases and the advantaged of these plants is been 100% natural with little or no side effects. Therefore a lot of studies have been done by researchers all over the world to determine the bioactive component in medicinal plant, which could replace artificial products or serve as lead compounds in drug design and synthesis of new and more effective drugs. Many researchers aimed to identify the possibility of using plants components to solve human health problems¹.

Various nutritional supplements from plants have been implicated as causing harmful effects². It is to be noted as well that nutritional supplements of the antioxidants comes from plant sources. These plants often exhibit a wide range of biological and pharmacological activities, such as anti-inflammatory, anti-fungal and anti- bacterial properties³. Plant foods especially tropical vegetables contain a large arrav of chemically diverse bioactive compounds with potential health promoting properties^{4, 5}. Leafy vegetables contribute to meeting the nutritional and medicinal needs of sub-tropical local populations especially in Nigeria as staples, flavours, condiments, spices, drinks and beverages in most developing countries⁶. Talinum triangulare is one of vegetables widely cultivated and consumed in Africa espe-cially Southern Nigeria⁷. Τ. triangulare is a cosmopolitan weed belonging to the Talinum genus, family Portulacaceae that grows best under humid conditions⁸. The leaves of T. triangulare is eaten as a vegetable and employed in the treatment of several disease conditions such as measles, sexually transmitted diseases and internal heat and is associated with varying biochemical and physiological effects⁹. The varying uses of the leaves of T. triangulare underscore its importance as major dietary supplement as a result of its nutritional and phytochemical composition¹⁰.

This is the basis of this preliminary study aimed at investigating the biochemical and antioxidant effects of *Talinum traiangulare* in female Sprague -Dawely rats.

MATERIALS AND METHOD Plant collection and extraction

Fresh leaves of *Talinum triangulare* were obtained from a farm in Itori-Ewekoro Local

Government Area, Ogun state. The fresh leaves were identified and given a voucher number of 110611 by Mr. Adeyemo of Forestry Research Institute of Nigeria (FRIN), Ibadan Oyo State.

The fresh leaves of *Talinum triangulare* were oven dried until constant weight was obtained. The air dried leaves were powdered using blender. The extract was filtered and evaporated at 40°c under reduced pressure. The yield of the dark browned colored dried extract obtained was 38.2%. The weighed extract was stored at 4°c until use in which case it was reconstituted in 1000ml of distilled water immediately before oral administration to the experimental animals.

Experimental animals

24 female Sprague- Dawley rats weighing between 150-200g were obtained from animal house of the College of Medicine of the University of Lagos, Idi-Araba. Experimental animals were kept in well-ventilated, hygienic compartments maintained under standard environmental conditions, acclimatized for three weeks before the experiment. The animals were well fed with standard rodent diet and water ad libitum. The experimental procedures adopted 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 at doses of 50mg/kg, 100mg/kg and 1500mg/kg body weight for fourteen (14) days.

Group A- Control (normal saline at 10mg/kg body weight).

Group B- 50mg/kg body weight.

Group C- 100mg/kg body weight.

Group D-150mg/kg body weight.

Collection of blood sample

Blood collection was done at the Research Laboratory of the Physiology Department of the University of Lagos. Blood Samples were collected from each rat as follows: 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. Once the sinus is puncture blood flows through the capillary tube and collected in an eppendorf tube. After the blood collection, the capillary tube is gently removed and wiped with sterile cotton and applying a little pressure with the collected blood samples were centrifuged fat 3000rpm for five minutes in order to get serum for biochemical and antioxidant analysis.

Biochemical analysis

Lipid profile analysis was carried out using an automated Analyzer (Roclie, HITACHI)

Antioxidant Studies

Superoixide Dismutase estimation was carried out using the RANSOD kit (Randox, Crumlin, England). Whole blood samples were used. The kit contained mixed substrate (xanthine, 0.05 mmol/L, and I.N.T. 0.05 mml/L), buffer (CAPS 40.00mmol/L, pH 10.2, EDTA, 0.94 mmol/L), xanthine oxidase standard (80U/L), sample diluents (5.40 U/L) and phosphate buffer (0.01mol/L, pH7.0) (50.00mL of 0.20 mol KH2 PO4 +29.65 mL of 0.20 N NaOH made up to 1L, with distilled water). The percentage inhibition of each sample was used to obtain the SOD units from a standard curve of the reconstituted and diluted RANSOD kit.

Glutathione peroxidase (GPx) was measured as follows,

0.05ml of heparinised whole blood was diluted with 1ml of diluting agent incubated for 5 minutes and 1ml of double strength hemoglobin reagent was added (i.e 1 volume of haemoglobin reagent diluted with 24 volumes of redistilled water). It was mixed well and assayed within 20 minutes of adding the haemoglobin reagent.

Phytochemical screening

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

Acute Toxicity test (LD₅₀)

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

Statistical Analysis

Data were recorded as mean and standard error of the Mean. Statistical difference between the means was determined by ANOVA. Any significant difference between means was assessed by the student's T-test and P<0.05 was accepted as the significant level.

Results

Table 1: Phase I of the acute toxicity (LD50) screening of the aqueous extracts of the leaves of *Telfairia occidentalis*

GROUPS (n=6)	DOSAGE (mg/kg)	MORTALITY
Group A	10	Nil
Group B	100	Nil
Group C	1000	Nil

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

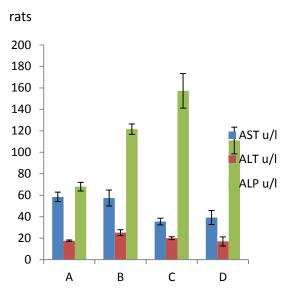
GROUP S (n=6)	DOSAGE (mg/Kg)	MORTALITY
Group A	1600	Nil
Group B	2900	Nil
Group C	5000	ST

ST= sign of toxicity

Phytochemical screening

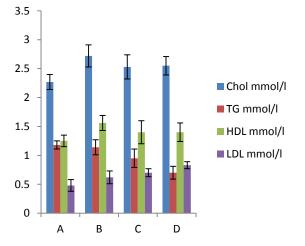
Results from the phytochemical screening of the plant showed the presence of the following phytochemicals; Molisch ++ Fehlings+ Flavonoid + Magnesium Chip++ Terpenoids + Glycosides ++ Tannins + Saponins ++

Figure 1: Effects of oral administration of aqueous leaf extract of *Talinum Triangulare* on liver function test in female Sprague -dawely

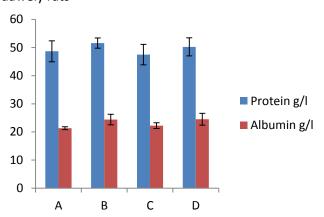


*Shows a significant interaction at p < 0.05 for AST, ALT and ALP, using student t-test. Group A: Normal saline (1ml/100 g of rat), B: (50 mg/1kg of rat) and C: (100mg/1kg of rat) .Group D (150 mg/1kg of rat).

Figure 2: Effects of oral administration of aqueous leaf extract of *Talinum Triangulare* on lipid profile female Sprague -dawely rats

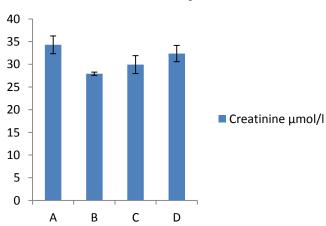


*Shows a significant interaction at p < 0.05 for CHOL, TG, HDL, LDL, UREA, CREAT, T-PROTEIN, ALB, AST, ALT and ALP, using student t-test. Group A: Normal saline (1ml/100 g of rat), B: (50 mg/1kg of rat) and C: (100mg/1kg of rat) .Group D (150 mg/1kg of rat). Figure 3: Effects of oral administration of aqueous leaf extract of *Talinum Triangulare* on protein and albumin levels female Sprague - dawely rats



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

Figure 4: Effects of oral administration of aqueous leaf extract of *Talinum Triangulare* on creatinine levels female Sprague -dawely rats



Creatinine µmol/l

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

The AST in group D was insignificantly reduced when compared with group A p>0.05. The ALT

level in group C (100ml/kg body weight) was significantly reduced and elevated in group D when compared with group A p>0.05. The ALP level was significantly increased when compared with group A, p<0.05 (figure 1). The CHOL level in group D was significantly increased when compared with the control group p>0.05. The TG levels significantly decreased in groups, B, C and D when compared with the control group (p>0.05). The result showed a significant decrease in HDL levels in all the tested groups except group D which showed significant elevation when compared with the control group p>0.05 (figure 2)

The protein levels were significantly increased in group B and significantly reduced in groups C and D when compared with the group A, P<0.05 (figure 3). The albumin level significantly increased in groups C and D when compared with the group A p<0.05 (figure 3)

The creatinine levels in figure 4 was significantly lowered in all the groups tested when compared with the group A (p>0.05).

Table 3: Effect of oral administration of aqueous leaf extract of *Talinum Triangulare* on antioxidant enzymes in female Sprague dawely rats

	А	В	С	D
SOD (mg/ml)	1.11±0.03	1.15±0.09	1.18±0.02*	1.07±0.06α
CAT (mg/ml)	7.25±2.35	8.48±0.46	7.31±0.29	9.39±1.09
GSH (µmol/ml)	599.62±24.38	701.53±37.77*	604.20±23.70#	776.35±90.38*α
MDA (nmol/ml)	8.74±0.07	8.54±0.14	8.66±0.10	8.59±0.06

*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), D (150 mg/1kg of rats)

The SOD was significantly elevated in group C and significantly reduced in group D when compared with group A, (p<0.05). The catalase was significantly elevated in all the tested groups B, C and D when compared with group A, (p<0.05). The GSH level was significantly increased in all the tested groups, B, C, and D when compared with the group A (p<0.05) while the MDA activity was significantly lowered in all the tested groups, when compared with group A p<0.05, (table 3).

DISCUSSION

Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds that are for biological functions, including defense against insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total.

Results from the present study revealed a decrease in lipid profile parameters majorly at the doses of 50mg/kg body weight and 100mg/kg, this shows that at these doses the aqueous leaf extract of Talinum triangulare level of cholesterol. Talinum reduced triangulare 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 $^{11, 12, 13, and 14}$. Thus, alterations in the concentration of the various lipid metabolism and predisposition of the heart to atherosclerosis and its associated coronary heart diseases.

However, it may cause liver damage when taken excessively as observed from the present study where at (150ml/kg body weight) there was increase in the level of liver enzyme activities. Furthermore, at all doses tested there was no significant increase in the level of kidney enzymes' activity and so no kidney damage was observed.

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. This study showed that SOD level was significantly increased (p<0.05) at 100mg/kg dose of extract. 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 flanovoid content¹⁴. *Mallotus oppsitifolium*, a Nigerian plant rich in flavonoids has been said to possess antioxidant as well as anti-inflamatory activities. The increase in antioxidant enzymes' activity observed showed the ability to fight diseases in the body.

Finally, since its activity is dose dependent care must be taken during its used as excessive intake can cause liver and kidney damage and also increase the level of LDL which is very detrimental to the body system.

From the present study, it is thus recommended that aqueous leaf extract of *Talinium triangulare* can be used as potent remedy for obesity. Since it is dose dependent, care should be taken during its therapeutic use.

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