

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND HYPOGLYCEMIC, HYPOCHOLESTEROLEMIC EFFECTS AND ACUTE TOXICITY STUDY OF AQUEOUS EXTRACT OF THE LEAF OF *TAPINANATHUS BANGWENSIS* IN EXPERIMENTAL WISTAR RATS.

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ABSTRACT: African mistletoe is an obligate parasitic medicinal plant found growing on evergreen deciduous plant. *Tapinanthus bangwensis* is a medicinal plant with wide-range spectrum of pharmacological potentials. Scientific investigations of the plant showed that it has anti-cancer, hypotensive, anti-infertility, anti-inflammatory, anti-oxidant properties etc. Within the scope of this scientific investigation of the aqueous extract of the leaf of *Tapinanthus bangwensis*, it could be deduced that the plant possesses both hypoglycemic and hypocholesterolemic properties and also has toxic tolerance. Phytochemical investigation of aqueous leaf extract of *Tapinanthus bangwensis* showed the presence of alkaloid, flavonoid, saponin, tannin, protein, carbohydrate, glycosides, cyanogenic glycoside, cardiac glycoside and steroid aglycon except reducing sugar that was absent. The percentage reduction in the level of systemic glucose compared to the control group was found to be 70%. Cholesterol level also reduced significantly by 39% compared with the control. Acute toxicity concentration was found to be 1833mg/kg. Statistical analysis showed significant difference at $p=0.05$.

KEYWORDS: *Tapinanthus bangwensis*, Reactive oxygen species, Acute toxicity, Diabetes mellitus, Phytochemical constituents.

INTRODUCTION

Investigative studies on the possible cure for diabetes mellitus and coronary heart related diseases is inexhaustible and as such researchers are looking for a permanent cure for this ugly menace that is ravaging the vast population of the world. Different group of oral hypoglycemic agents or drugs are currently available with characteristic profile of side effects (Zia-UI-Hag M et al., 2014). The search for anti-diabetic and hypotensive agents with little or no side effects is a continuous process. The plant kingdom is a field for effective oral hypoglycemic and hypolipidemic agents. One of such proven medicinal plant, potent for the treatment of these diseases is African mistletoe specifically *Tapinanthus bangwensis*. Diabetes mellitus is a chronic metabolic disorder that affects human body (Shazia Anwer Bukhari., 2014, Zia-UI-M et al., 2014, Muhammad T.S., 2014). Diabetes mellitus is a disease condition characterized by high sugar level in the systemic circulation due to lack of insulin (Type 1) or low production of insulin (Type 2) by the β -pancreatic cells found in the islet of Langerham. Thus this medical disease condition can also be called hyperglycaemia. Diabetes mellitus is observably evidence in the high volume of urine one excretes (WHO, 1980, Warjeet Singh, 2011). Diabetes mellitus is a 3rd killer disease along with cancer and cerebrovascular diseases (Chauhan et al., 2010). Diabetes mellitus is associated with microvascular and macrovascular complications which are the major causes of morbidity and death (Baynes JW, 1991). Coronary heart related diseases such as heart failure, stroke, paralysis,

hypertension, Artherosclerosis, hyperlipidemia etc occurs as a result of high fat dietary intake (Obatomi et al, 1996). Statistical reports and health prediction showed that by 2030 the mortality rate from these diseases would increase astronomically and this calls for radical or urgent measures to avert this menace. So many factors have been attributed to the cause of diabetes mellitus and coronary heart diseases, some of such factors include poor dietary intake, disease disorders, metabolic disorders etc. Among many causative agents associated with diabetes mellitus and coronary heart disease, oxidative stress has also been fingered. Oxidative stress is associated with increase production of reactive oxygen species (ROS) which can cause oxidative damage of the insulin and adipose-receptor cells thereby reducing sugar and lipid absorption respectively (Bagria et al, 2009).

Tapinanthus bangwensis is one of the species of African mistletoe belonging to the family of Loranthaceae. It is an obligate parasitic plant found growing on deciduous trees in ball-like bush. *Tapinanthus bangwensis* is a photosynthetic plant that can convert solar energy into chemical form in form of food (Evans J, 2005, Osadebe and Uzochukwu, 2006). Research reports showed that *Tapinanthus bangwensis* is anti-diabetic and anti-hypertensive agents (Kafaru, E, 1993), anti-cancer agent (Grossarth-Maticek, R, Ziegler R, 2007), anti-microbial agent, fertility enhancer etc. The aim of this study was to ascertain the hypoglycemic and hypocholesterolemic properties and acute toxicity of aqueous extract of the leaf of *Tapinanthus bangwensis* in experimental wistar rats.

MATERIALS AND METHODS

Tapinanthus bangensis plant was purchased from Mushin market in Lagos State and was botanically identified by Mr Adeleke in the department of Pharmagnosy, College of medicine, Idi-Araba, Lagos State.

Preparation of plant extract:

The leaves of *Tapinanthus bangwensis* were removed from the twigs and sun-dried for days. The dried leaves were then grounded into powdered form using mortar and pestle. Aqueous extraction method was used. 50g of the powdered leaf was dissolved in 800ml of distilled water and left for 3days and then filtered with No.1 whatman paper. The filtrate was then concentrated by evaporation process.

Animals:

35 wistar rats were obtained weighing 100-120g from the Animal Laboratory Center, University of Lagos, Lagos State. The genetically modified rats were then acclimatized for two weeks and were fed with nutritionally formulated rat feed and distilled water.

Phytochemical Evaluation:

The Phytochemical compositions of *Tapinanthus bangwensis* were determined by using the procedure of Evans, 2005.

Alkaloid Test:

0.5g of each extract was boiled with 5ml of 2% HCL on a water bath. 1ml portion of each filtrate was treated with 2 drops of Mayers reagent (mixture of 36g of HgCL, 5g of KI and 100ml distilled water) and a cream coloured precipitate was observed indicating the presence of alkaloid.

Flavonoid Test

0.5g of each extract was heated with 10ml ethyl acetate in a boiling water for 3mins. 4ml of each filtrate was shaken with 1ml of 1% $AlCl_3$ solution, a light yellow coloration in ethyl acetate layer was observed showing the presence of flavonoid.

Saponin Test (frothing test)

Each extract of the plant was boiled with 5ml of distilled water. 1ml of the filtrate was diluted with 4ml of distilled water. On shaking vigorously, a stable froth was observed after a while indicating the presence of saponins.

Tannin Test (Hydrolyzable test)

0.5g of each extract was boiled in 10ml of distilled water for 5mins and then filtered. The filtrates were added a drop of bromine water and an orange precipitate was observed.

Protein Test

5ml of distilled water was added to some quantities of the extracts and were allowed to stand for 3hrs. 2ml of the solution was added to 0.1ml of millon reagent (mixture of $HgNO_3$ and HNO_3). On shaking vigorously, a yellow precipitate was observed showing the presence of protein.

Carbohydrate Test (Molisch Test)

0.5g of each plant extracts was added water. Two drops of molisch reagent (mixture of 15g of α -naphatol and 100ml of chloroform) was added to the solutions and shaken vigorously. 2ml of 10% concentrate H_2SO_4 was carefully added and a layer was formed below the solution forming a deep violet coloration at the interface, indicating the presence of carbohydrate.

Reducing Test (Fehling Test)

0.5g of each extract was added 5ml of distilled water. 5ml of both Fehling A (17.3g of $CuSO_4$ dissolved in distilled water and made up to 250ml) and Fehling B (86.5g of sodium potassium tartrate was dissolved in warm water, 30g of NaOH was dissolved in distilled water and both solutions were then added and volume made up to 250ml) was added to the solutions. On vigorously shaken, a brick-red precipitate was observed indicating the presence of reducing sugars.

Steroidal Aglycon (Salkowski Test)

0.5g of each extract were dissolved in 2ml of chloroform. 10% concentrated H_2SO_4 was carefully added to form a lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring that is aglycon portion of the cardiac glycosides.

Cardiac glycosides (Keller- Killian Test)

0.5g of each extract was dissolved in 2ml of glacial acetic acids containing few drops of 10% FeCl₃ solution. This was then underplayed with 1ml of 10% of concentrated H₂SO₄. A brown ring obtained at the interface indicates the presence of deoxy sugar characteristic of cardenoides.

Cyanogenic glycosides Test

0.5g of the plant extracts were put into a conical flasks and 20ml of distilled water was added to cover the extracts. A piece of sodium picrate paper was suspended in the flask on a water bath for 1hour. A colour change from yellow to orange was observed showing the presence of cyanogenic glycosides.

Experimental design:

Group A : Control group placed on distilled water only.

Group B: Rats fed orally with 100mg/kg aqueous leaf extract of *Tapinanthus bangwensis*.

Group C : Rats fed orally with 200mg/kg aqueous leaf extract of *Tapinanthus bangwensis*.

Group D : Rats fed orally with 300mg/kg aqueous leaf extract of *Tapinanthus bangwensis*.

Group E : Rats fed orally with 400mg/kg aqueous leaf extract of *Tapinanthus bangwensis*.

Preparation of Animals

The rats were administered the aqueous extract of *Tapinanthus bangwensis* orally for seven (7) days. They were fasted overnight and then sacrificed by capitulation. Blood samples were collected from the heart into Lithium bottles and labelled correctly. The blood samples were centrifuged at 3000rpm and the serum used for laboratory analyses.

Determination of glucose concentration:

The glucose oxidase method by Sharma et al., 1997 was used.

Procedure:

Get five test tubes. One test tube the blank (or control) while the other test tubes for the samples.

To the blank test tube add 2000µl of the reagent mixture only while the sample test tubes contains 20µl of the samples and 2000µl of reagent mixture.

Mix the content of each test tubes thoroughly and incubate at 37°C

Take absorbance at wavelength of 500nm.

Glucose concentration in the sample

$$= \frac{A_{\text{sample}} \times C_{\text{standard}}}{A_{\text{standard}}}$$

Where A = Absorbance, C = Concentration

Determination of cholesterol concentration:

The cholesterol oxidase/peroxidase method of Abell et al., 1952 was adopted.

Procedure:

Get test tubes and label them as Blank (control), standard and samples

To the blank test tube add 10µl of distilled water, to the standard test tube add 10µl of the standard while the sample test tube contain 10µl of the sample.

To each of the test tubes, add 1000µl of the reagent mixture.

Mix the content of each test tube thoroughly and incubate at 37°C

Take absorbance at wavelength of 500nm.

Cholesterol concentration in the sample

$$= \frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}}$$

Where A = Absorbance, C = Concentration

Acute Toxicity Test in Rats:

The procedure of Finney, 1964 was adopted. Different concentrations of the aqueous plant extract were prepared: 10mg/kg, 100mg/kg, 1000mg/kg, 5000mg/kg and 10000mg/kg respectively and were administered intraperitoneally to each group, A, B, C, D and E respectively.

Statistical analysis:

The data obtained were analysed using Mean \pm SD and ANOVA at confidence limit of 95% ($p = 0.05$).

RESULTS

TABLE 1: PHYTOCHEMICAL SCREENING RESULT:

Phytochemical constituents	Presence/ Absence
Alkaloid	+
Flavonoid	++
Saponin	++
Tannin	+
Protein	+
Carbohydrate	++
Reducing sugar	-
Glycosides	+
Cyanogenic glycoside	+
Cardiac glycoside	+
Steroid aglycon	+

+ = Present, - = Absence, ++ = Present in higher concentration.

TABLE 2: Effect of aqueous extract of leaf of *T. bangwensis* on serum glucose (mmol^{-1}) administered at various concentrations.

Groups	No of rats	Dose (mg/kg)	Mean \pm SD
A	4	Control	5.90 \pm 0.23
B	4	100	3.20 \pm 0.50
C	4	200	2.20 \pm 0.14
D	4	300	2.20 \pm 0.14
E	4	400	1.75 \pm 0.33

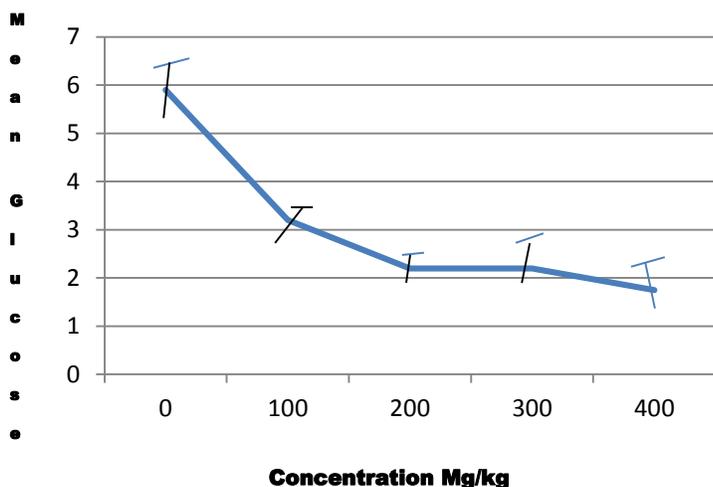


Figure 1: Effect of aqueous extract leaf of *Tapinanthus bangwensis* on systemic glucose in wistar rats

TABLE 3: Effect of aqueous extract of leaf of *T. bangwensis* on serum cholesterol (mg/dL) administered at various concentrations.

Groups	No of rats	Dose (mg/kg)	Mean \pm SD
A	4	Control	171.7 \pm 0.0
B	4	100	158.3 \pm 0.24
C	4	200	143.0 \pm 0.15
D	4	300	108.3 \pm 0.40
E	4	400	104.2 \pm 0.24

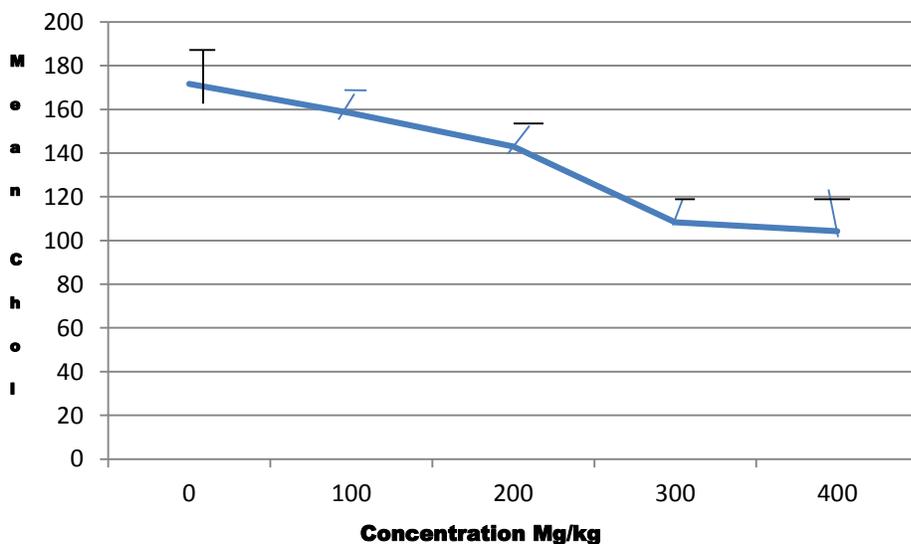


Figure 2: Effect of aqueous extract leaf of *Tapinanthus bangwensis* on systemic cholesterol in wistar rats.

TABLE 4: Acute toxicity of aqueous extract of *T.bangwensis* on wistar rats administered at varying concentrations.

Group	No of rats	Dose administered	Dose difference (a)	Log dose(mg /kg)	No alive	No dead	Mean mortality (b)	% dead	Probit value (a x b)
A	3	10	-	1	3	0	0	0	0
B	3	100	90	2	3	0	0	0	0
C	3	1000	900	3	3	0	0	0	0
D	3	5000	4000	3.7	2	1	0.5	33	2000
E	3	10000	5000	4	1	2	1.5	66	7500

LD₅₀ = Least lethal dose – $\frac{\sum a \times b}{N}$ where N= No of rats in each group

$$\begin{aligned}
 &= 5000 - 9500/3 \\
 &= 5000 - 3167 \\
 &= 1833\text{mg/kg.}
 \end{aligned}$$

DISCUSSION

Medicinal value of plants lies in the bioactive phyto-components of the plants (Veermuthu et al., 2006). From the phytochemical evaluation of aqueous extract leaf of *T.bangwensis* using the method of Evans, the following phytochemical materials were observably present, saponin, Tannin, glycosides, alkaloids, steroidal aglycon, protein, carbohydrate. From phytochemical analysis as shown in Table 1, the presence of flavonoid, saponin and carbohydrate were found to be higher compared to other phytochemical constituents. Within the limit of the phytochemical investigation, it was observed that only reducing sugar was absent. The phytochemicals that exhibit pharmacological effects in aqueous extract of *Tapinanthus bangwensis* are Saponin, Flavonoid and Tannin.

The percentage reduction in the level of glucose as shown in Table 2, was found to be 45.7%, 62.7%, 62.7% and 70.3% respectively compared with the control. Fig 1, showed a significant decrease in systemic glucose. Also, in fig 1, it was observed that an increase in the drug concentration from 200mg/kg to 300mg/kg showed no significant decrease in glucose level, this was attributed to the fact that the glucose level in the rats have reached its normoglycemic state. The hypoglycemic effect of the aqueous extract of *Tapinanthus bangwensis* could either be by increasing the peripheral utilization of glucose or by stimulating the secretion of insulin by the β -pancreatic cells (Obatomi et al., 1997; Nwaegerue et al., 2007).

From the cholesterol investigation and given by the result shown in Table 3, the percentage reduction effects of aqueous extract of *Tapinanthus bangwensis* compared with the control were found to be 7.8%, 16.7%, 36.9% and 39.3% respectively. From fig 2, the sharpest decrease in the concentration of serum cholesterol was observed at concentration between 200mg/kg and 300mg/kg. In fig 2, it was also observed that an increase in dose from 300mg/kg to 400mg/kg showed slight decrease in cholesterol level, although the decrease was not very significant.

The cholesterol lowering action of the aqueous leaf extract of the plant may have been due to the presence of saponin. This effect is achieved by the binding of bile acids and cholesterol by saponin thereby forming strong insoluble complexes with cholesterol. Saponin causes a depletion of cholesterol preventing it from reabsorption, thus increasing its excretion (Iheanacho et al., 2008). The cholesterolemic effect of aqueous extract of *Tapinanthus bangwensis* in the experimental rats could be that the extract could have stimulated or activated the hepatic cells for the secretion of bile for fat emulsification and later degraded by lipase to produce energy for biological activities (Matheson HB et al., 1995, Everson GT et al., 1992).

Mistletoe is toxic tolerant. From the acute toxicity study according to Table 4, it would be observed that the LD₅₀ was 1833mg/kg, which implies that at this concentration, fifty percent of the animal population can be killed. Mistletoes could be considered to be toxic probably because of some of their constituents such as lectins which are cytotoxic to certain organisms. African mistletoes were described as toxic berries (Adodo, 2004) which lead to death by heart attack when consumed in large quantities (Dibong et al., 2009).

CONCLUSION:

From the research findings, it can be concluded that aqueous extract of the leaf of *Tapinanthus bangwensis* was a very potent hypoglycemic and hypocholesterolemic agent but it has more hypoglycemic property than hypocholesterolemic property. It was also found that aqueous extract of the leaf of *Tapinanthus bangwensis* was toxicologically safe in rats.

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