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Tapinanthus bangwensis (Mistletoe) reduces some increased lipid profile parameters caused by alloxan-induced diabetes in Wistar Rats

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Abstract: **This study investigated the effect of aqueous extract of Tapinanthus bangwensis (T. bangwensis) on some lipid profiles in alloxan-induced diabetic Wistar rats. The effects of this plant extract were monitored on the serum concentrations of triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C) and cholesterol (CHOL). Sixty-six albino rats were used for the study arranged into eleven groups of six rats each. Two groups (12 rats) were used for a pilot study. Nine other groups (54 rats) were used for the experiment labeled groups 1-9. Group 1 constitutes the normal control which received only feed and water, group 2 received 50mg/kg citrate buffer. Alloxan was dissolved in 0.1M citrate buffer solution, pH 4.5. Group 3 received 50mg/kg alloxan dissolved in citrate buffer in a 1:1 ratio intraperitoneally. Groups 4-6 were treated with 50mg/kg citrate buffer with 5%, 7%, and 10% aqueous extract of T. bangwensis respectively. Groups 7-9 received 50mg/kg alloxan dissolved in citrate buffer in a 1:1 ratio intraperitoneally with 5%, 7%, and 10% aqueous extract of T. bangwensis respectively. Blood samples were collected into appropriately labeled sampled bottles and analyzed. Results of the blood analysis showed that alloxan caused diabetes mellitus in the experimental animals. Lipid profile parameters such as triglyceride (TG) (0.820±0.01), high-density lipoprotein cholesterol (HDL-CHOL) (0.480±0.00), and cholesterol (CHOL) (2.100±0.06) of the control animals were found to be within the normal range. These parameters significantly increased in the animals induced with diabetes but not treated. Treatment with the plant extract sparingly reduced the raised serum triglycerides in a dose-dependent manner. This study was able to establish the diabetogenicity of alloxan as seen in lipids, concentrations are elevated and treatment with Tapinanthus bangwensis reduced the raised serum triglycerides.**

***Keywords: Tapinanthus bangwensis*, Mistletoe, serum lipid metabolites, elevated parameters, lipid profile, Alloxan, diabetes, Wistar rats.**

1. INTRODUCTION

According to the World Health Organization, at least 171 million individuals globally, or 2.8% of the population, have diabetes as of 2000 (Wild et al., 2004). It is predicted that this figure would nearly double by the year 2030 due to its significant increase in incidence. Diabetes mellitus is a disease that affects people everywhere, however, it is more prevalent (particularly type 2) in developed nations.

However, it is predicted that prevalence would rise most dramatically in Asia and Africa, where the majority of patients will likely be found by 2030 (Wild et al., 2004). The trend of urbanization and lifestyle changes, probably most crucially a "Western-style" diet, is related to the rise in diabetes incidence in developing nations. Insulin, a hormone made in the pancreas, is a hormone that the body either does not make enough of or does not respond to correctly in people with diabetes

mellitus. Cells can take up glucose and use it as fuel thanks to insulin. This results in an accumulation of glucose in the blood (hyperglycemia), which can induce a number of possible consequences (Rother, 2007; Tierney et al., 2002).

There are many different types of diabetes, but the main three are Type 1 diabetes, which develops when the body is unable to produce insulin, Type 2 diabetes, which develops when cells are unable to properly use insulin, sometimes in conjunction with absolute insulin deficiency (Tierney et al., 2002), and gestational diabetes, which affects about 4% of all pregnant women (Tierney et al., 2002).

Without the right care, diabetes can lead to numerous complications. Hypoglycemia, diabetic ketoacidosis, or non-ketotic hyperosmolar coma are examples of acute consequences. Cardiovascular disease, chronic renal failure, and retinal damage are serious long-term consequences. Thus, proper diabetes management is crucial, along with blood pressure management and lifestyle choices including quitting smoking and keeping a healthy weight (Elliot et al., 1996).

In many underdeveloped nations, the foundation of medical care is provided by medicinal plants (Eisenberg et al., 1998; Gray and Flatt, 1999). In addition to being used as food, several plants are also recognized to have medical uses (Orhue et al., 2008; Uahomo et al., 2022). Despite the widespread belief that plant-based natural medicines are safe, scientists advise conducting adequate toxicological research (Orhue et al., 2008; Oyewole et al., 2007) to guarantee the safety of natural medicine use.

Omeodu and his colleagues have extensively researched a number of plants for their potential anti-diabetic properties (Omeodu et al., 2023; Omeodu et al., 2022; Akoko et al., 2022), however, none of these plants were parasitic in nature. Most communities in Nigeria employ the parasitic plant *Tapinanthus bangwensis*, sometimes known as mistletoe, to cure and control diseases like diabetes, high blood pressure, asthma, epilepsy, and cancer (Ekhaise et al., 2008). This study was carried out to find out how various lipid profiles in Wistar Rats with alloxan-induced diabetes will respond to an aqueous extract of *Tapinanthus bangwensis* (Mistletoe).

2. MATERIALS AND METHODS

Preparation of plant materials

The plant used for this work is *T. bangwensis*. The plant was obtained at the University of Port Harcourt where it was found hemi-parasitizing on a specie of orange (*Citrus quarantum*) orchard located on the right side of the front of the Vice-Chancellors lodge at the Delta Park of the university. The leaves which were used for this work were carefully plucked off, thoroughly washed, and air-dried for twenty-four (24) days until a constant weight was obtained.

Preparation of aqueous *T. bangwensis* extract

T. bangwensis was collected with stalks. The fresh greenish leaves were carefully plucked from the stalk and the pedicels were removed from each leaf. The leaves were thoroughly washed and spread out on clean cardboard paper and kept at room temperature in a well-aerated room. They were allowed to dry to constant weight after twenty-four days (24). The dried sample was then pounded in a mortar with a pistol. After pounding, the partially powdered sample was grounded with a manual grinding machine until a fine powder was obtained. Fifty grams of the powdered mistletoe was measured and dissolved in a 1-liter measuring cylinder containing 500ML distilled water. The mixture was then stored at room temperature for twenty-four (24) hours (Omeodu *et al.*, 2008).

The preparation was then filtered using ten different pieces of white cloth. The filtrate was filtered two times through a Whatman No.541 filter paper and the stock was stored in a refrigerator at a temperature of 40°c for 24 hours. 50mg/kg, 70mg/kg, and 100kg of the filtrate were then prepared from the stalk solution and these three different concentrations were used to treat the test animals (Omeodu *et al.*, 2008).

Test animals

The animals were divided into experimental groups of six (6) animals per group and each group was housed in a metabolic cage. They were provided with feeds and water to the libitium. The animal feeds were purchased from the livestock feeds, Choba, a division of livestock feeds Nig. Ltd. Ikeja, Lagos, while the water was supplied by the Water Treatment Plant, Choba Park, University of Port Harcourt. There were a total of nine (9) experimental groups. All the rats weighed between 200g to 300g and their average was fourteen months.

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Experimental protocol

The investigated animals consisted of nine groups with six animals per group (the experimental design is shown below). Each animal was labeled with picric acid for easy identification on the head (HD), right (RH), left hands (LH), left leg (LL) and tail (TL).

Table 1: Experimental Protocol

Group	Identification	Administration
Group 1	Normal control	Feed and water only.
Group 2	Normal control 2	Feed, water and citrate buffer solution
Group 3	Normal diabetic control (NDC)	Feed, water and alloxan solution
Group 4	Normal treated control (NT-1)	Feed, water, citrate buffer and 5% *T. bangwensis* solution
Group 5	Normal treated control (NT-2)	Feed, water, citrate buffer and 7% *T. bangwensis* solution
Group 6	Normal treated control (NT-3)	Feed, water, citrate buffer and 10% *T. bangwensis* solution
Group 7	Diabetic treated control (DT-1)	Feed, water, alloxan solution and 5% *T. bangwensis* solution
Group 8	Diabetic treated control (DT-2)	Feed, water, alloxan solution and 7% *T. bangwensis* solution
Group 9	Diabetic treated control (DT-3)	Feed, water, alloxan solution and 10% *T. bangwensis* solution

Group one animals were administered only feeds and water *ad libitum* to serve as the general control group. Group two animals received citrate buffer solution in addition to feeds and water. Alloxan solution was administered to a group of three animals and allowed free access to feeds and water. Before citrate and alloxan administration to groups two and three respectively, the animals fasted for eighteen (18) hours. This was the same for all groups of four to nine animals which received various treatments. Groups four to six were administered with citrate buffer at 50mg/kg dose, while groups seven to nine animals were administered with alloxan solution at the same 50mg/kg and then treated with aqueous *T. bangwensis* solution at a dose of 250mg/kg with group seven receiving 50mg/kg of the *T. bangwensis* extracts, group eight receiving 70mg/kg and group nine receiving 100mg/kg of the extract.

Administration of *T. bangwensis* extract

The *T. bangwensis* stock solution was prepared into 5%, 7% and 10% by the process already stated by Omeodu *et al.*, (2008). These three different preparations were fed only to group 4, 5 and 6 respectively, at a dose of 250mg/kg body weight of animal on daily basis. The treatment continued for twenty-one (21) days at the end of which all the nine groups were sacrificed by cervical dislocation method and their whole blood collected for analyses. Each of the animal's pancreas and their liver were also collected and preserved in 10% formaldehyde.

Sample collection for analyses

At the end of the twenty-one days of extract administration, the animals were sacrificed on the twenty-second day. Each rat to be sacrificed was withdrawn from its cage and anesthetized in a chloroform saturated chamber (Omeodu *et al.*, 2008). The blood sample was the collected from the animal after withdrawing from the chamber by cardiac puncture into appropriately labeled sampled bottles, its pancreas and liver tissues was also collected into separate sample bottles and preserved in formaldehyde. These samples at the end of the collection were quickly taken to the laboratory for analysis. The blood specimens were centrifuged at 5000rpm using MSE centrifuge to obtain plasma. The liver and pancreatic samples were prepared into slides and analyzed at the anatomical histopathology laboratory of the University of Port Harcourt teaching hospital.

Biochemical analysis

Serum cholesterol was estimated according to the method described by Trinder, (1969), and serum triglycerides were estimated according to the method described by (Lothar, 1998). HDL-Cholesterol and LDL-Cholesterol was estimated according to the method described by Jacobs et al., 1990.

Statistical analysis

The data was statistically analyzed using GraphPad Prism Software (2000) version 3.05 by GraphPad Inc. Data are presented in Mean±Std. Statistical significance was accepted at a level of $p<0.05$ and below

3. RESULTS

The results of the investigations shown in Table 2 indicated clearly that alloxan caused diabetes mellitus in the experimental animals. Values of the control animals were found to be within the normal range for the parameters analyzed. Treatment of the plant extract sparingly reduced the raised serum triglycerides in a dose-dependent manner.

Table 2: This shows mean serum lipids levels in alloxan-induced diabetic rats treated with mistletoe extracts

Group	TG	HDL-CHOL	CHOL
1	0.820 ± 0.01^{c}	0.480 ± 0.00^{a}	2.100 ± 0.06^{bcd}
2	1.220 ± 0.03^{a}	0.530 ± 0.01^{a}	2.300 ± 0.05^{bc}
3	1.222 ± 0.02^{a}	0.471 ± 0.02^{a}	2.704 ± 0.04^{a}
4	0.870 ± 0.01^{c}	0.452 ± 0.01^{a}	2.080 ± 0.03^{cd}
5	0.850 ± 0.01^{c}	0.520 ± 0.01^{a}	1.920 ± 0.01^{d}
6	0.930 ± 0.02^{bc}	0.571 ± 0.02^{a}	1.952 ± 0.04^{cd}
7	1.250 ± 0.04^{a}	0.570 ± 0.02^{a}	2.451 ± 0.07^{ab}
8	1.270 ± 0.03^{a}	0.570 ± 0.01^{a}	2.232 ± 0.02^{bcd}
9	1.12 ± 0.05^{ab}	0.570 ± 0.02^{a}	1.970 ± 0.03^{cd}

4. DISCUSSION

Traditional plants have been used for ages in the management and treatment of diabetes mellitus but only a few of them have been recommended (Kafaru, 1994). This work, therefore, exposes the need to thoroughly subject African medicinal herbs to scientific scrutiny. Diabetes mellitus has for many years evaded comprehensive management and treatment using orthodox drugs, and where they tend to succeed the attendant clinical side effects oftentimes overshadow the success.

Swanston-Flatt et al (1989) reported that many clinical parameters associated with diabetes mellitus in experimental animals were reduced when administered with an extract of mistletoe (6.25%) by weight. He observed that such diabetes-associated symptoms as polydipsia, polyphagia, and body weight loss were all ameliorated with mistletoe administration. The work of Obatomi et al. (1994) has lent credence to this present work. In this work, it was observed that alloxan given to experimental rats at a dose of 50mg/kg induced diabetes mellitus in them. Only a slight reduction was observed in the elevated serum cholesterol of 27%. Hyperlipidemia due to high serum TG or total cholesterol concentration or both has been reported in diabetic and hypertensive patients (Nikkila, 1984).

5. CONCLUSION

This study was able to establish the diabetogenicity of alloxan as seen in the glucose level, and that in lipids, concentrations are elevated. Therefore, this work has shown that extract of African *Tapinanthus bangwensis* is insulinogenic and thus, can be a good anti-diabetic agent as it can improve most of the altered biochemical and physiological parameters observed during diabetes mellitus.

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