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Evaluation of the Methanolic Extract of Mistletoe (*Tapinanthus bangwensis*) Leaves Grown on Orange Trees for the Phytochemical Properties and its Physiological Effects on Streptozotocin Induced *Diabetes mellitus* in Laboratory Animals

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Abstract: Mistletoe (*Tapinanthus bangwensis)* a semi-parasitic evergreen plant, that has been used traditionally in Nigeria and other parts of Africa as antihypertensive and antidiabetic agents. The aim of this research was to investigate the phytochemical properties of methanolic extract of *Tapinanthus bangwensis* (mistletoe) and its physiological effects on sugar levels in laboratory animals. The phytochemical analysis revealed the presence of saponins, flavonoids, tannins and steroidal glycosides. Treatment with aqueous *Tapinanthus bangwensis* (mistletoe) extract at the dose of 500mg/kg body weight showed that the cocnentration of blood glucose levels in the diabetic test (treated rats) were significnatly reduced as compared to the diabetic control (untreated rats). In streptozotocin induced diabetic experimental animals (rats), maximum reduction in blood glucose levels was observed after fourteen (14) days of treatment with methanolic crude extraxt of *Tapinanthus bangwensis* (mistletoe). The result showed the concentrations of blood glucose in the diabetic test (treated) group was significantly reduced to 163.75 ± 46.327 ($P < 0.05$) (mg/dl) after fourteen (14) days of administration of aqueous *Tapinanthus bangwnesis* (mistletoe) extract at 500 mg/kg body weight as compared to 377.50 ± 0.50(mg/dl) of the diabetic control (untreated) group. The result indicated that the methanolic crude extract of *Tapinanthus bangwensis* leaves possess significant anti-diabetic activity.

Key words: Methanolic extract of mistletoe · Experimental animals · Streptozotocin · *Diabetes mellitus.*

INTRODUCTION

The term *diabetes* was coined by Aretaeus of Cappadocia and it was first recorded in English, in the form diabete, in a medical text written around 1425. In 1675, Thomas Willis added the word *mellitus*, from the Latin meaning "honey", a reference to the sweet taste of the urine. This sweet taste had been noticed in urine by the ancient Greeks, Chinese, Egyptians, Indians and Persians [1].

Diabetes mellitus was seen as death sentence in the ancient era. Aretaeus did attempted to treat it, but could not give a good prognosis, hence it was seen that "life with diabetes is short, disgusting and painful "[1].

In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of the world population [2]. Its incidence is increasing rapidly and it is estimated that by the year 2030, this number will almost double. *Diabetes mellitus* occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 [2]. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet.

Diabetes mellitus is a condition in which the body either does not produce enough or does not properly respond to insulin, a hormone produced in the pancreas. Insulin enables cells to absorb glucose in order to turn it into energy. This causes glucose to accumulate in the blood (hyperglycemia), leading to various potential complications [3, 4].There exists a number of diabetes, but the principal three major types are Type 1 diabetes which results from the body's failure to produce insulin.

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Presently most persons with type 1 diabetes take insulin injections. Type 2 diabetes results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with absolute insulin deficiency [4]. Pregnant women who have never had diabetes before but who have high blood sugar (glucose) levels during pregnancy are said to have gestational diabetes. Gestational diabetes affects about 4% of all pregnant women [4]. It may precede development of type 2 (or rarely type 1). Other forms of diabetes mellitus includes congenital diabetes due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids and several forms of monogenic diabetes. All forms of diabetes have been treatable since insulin became medically available in 1921, but a cure is difficult [3]. Diabetes without proper treatments can cause many complications. Acute complications include hypoglycemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure and retinal damage. Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as smoking, cessation and maintaining a healthy body weight [5].

The use of herbal medicine in health treatment is gaining considerable recognition and popularity worldwide [6]. Despite much skepticism to support its therapeutic efficacy, the use of herbal remedies has increased to about 38 percent during the last 7 years in the United States [7]. Before the introduction of insulin in 1922, the treatment of *Diabetes mellitus* relied heavily on dietary measures which included the use of traditional plants therapies. Many traditional plant treatments for diabetes exist [8], however, few have received scientific or medical scrutiny and the World Health Organization has recommended that traditional plant treatment for diabetes warrant further evaluation [9]. Medicinal plants form the basis of medical treatment in many developing countries [7, 8]. Some plants, apart from serving as food, have also been known to exhibit medicinal properties [10]. Although plant based natural medicines are popularly accalaimed to be safe, scientists advocate for proper toxicological studies [10, 11] in other to ensure safety in the use of natural medicines. *Tapinanthus bangwensis* (Mistletoe) is a parasitic plant grown citrus plant and a variety of other plants [6], used by most communities in Nigeria for the treatment and management of ailments such as diabetes, blood pressure, asthma, epilepsy and cancer. This study was undertaken to investigate the phytochemical properties of methanolic extract of mistletoe (*Tapinanthus bangwensis)* grown on Orange trees and its physiological effects on sugar levels (*Diabetes mellitus*) in laboratory animals (rats).

MATERIALS AND METHODS

Collection and Extraction of Plant Materials (Misstletoe): The leaves of mistletoe plants *(Tapinanthus bangwensis)* used for the research work were obtained from orchards of orange trees (*Citrus sinensi*) at the University of Benin, Faculty of Social Sciences Garden, upon collection the plants were taken to Department of Pharmacognosy, Faculty of Pharmacy and University of Benin, Benin City for identification.

The mistletoe plants were dried and leaves removed from the whole plant. The leaves were grinded under aspetic conditions into fine powder form for phytochemical and microbiological experimentations.

Five hnudred grams (500g) of fine powder form of mistletoe was weighed into a clean glass ware containing 2000ml or 2l of methanol (extracting solvent). The mixture was thoroughly and vigorously shaken in order to have a proper mixing and extraction by the solvent. This was carried out for 3days (72 hours), after which the mixture was filtered to obtain a crude filtrate. The filterate was concentrated using a water bath to evaporate the extracting solvent (methanol) and later transferred to hot oven, to evaporate any trace of the solvent. Upon evaporation, the filtrate was steamed-dried and weighed. This was preserved for further biological physiological and physiochemical analysis.

Phytochemical Screening of Methanolic Extract of Mistletoe *(Tapinanthus bangwensis):* The screening procedure was carried out according to the standard methods of Sofowora [13].

Experimental Animals and Treatment: Sixteen albino Wistar rats, weighing between 125 and 160 were obtained from the National Institute of Medical Research (NIMR), Yaba, Lagos. The animals were divided into 4 groups, of 4 animals in each group. The animals were maintained under standard environmental conditions in the Animal House, Department of Biochemistry, Faculty of Life Sciences University of Benin, Benin City. They were fed with normal standard diets obtained from Bendel Feeds and Flour Mill, Ewu, Edo State, without any restriction to food and drinking water during the experimental period. After two (2) weeks of acclimatization, eight (8) rats were injected with the diabetogenic drug, streptozotocin (STZ).

One hundred miligram (100mg) of the drug was dissolved in 2ml 0.9% Normal saline (pH = 4.5). The stock solution was administered to each rat at a dose of 60mg/kg body weight with the aid of a hypodermic needle intraperitoneally.

Group 1: This group serves as control that received feed + distilled water.
Group 2: The diabetic test group that receives feed + distilled water + STZ + plant extracts.
Group 3: The labelled diabetic test control group receives feed + distilled water + STZ.
Group 4: (Extract control) receives feed + distilled water + extract.

Animals in the test and extract control groups (II and IV) were orally administered the aqueous extract of *Tapinanthus bangwensis* once daily over a period of 14 days at a dosage of 500mg/kg body weight.

Preparation of Serum and Biochemical Assays: At 24 hours after the last administration of extract, the animals were anaesthetized under chloroform vapour and dissected. Blood for serum preparation was collected by cardiac puncture, using sterile syringes into sterile plain tubes without anticoagulant. The serum was harvested for the clot by centrifugation at 2000 rpm for 10 minutes using a bench top centrifuge into sterile plain tubes. The serum was stored in the refrigerator for the analysis of biochemical parameters.

The blood glucose level was assayed using a commercial Glucometer (Accu-CHECK) and Strips. Serum was separated and blood glucose levels were measured immediately by glucose-oxidase method [14]

Statistical Analysis: Results were expressed as means ± S.E.M. The data were analysed by exploring the student's unpaired t-test and one-way analysis of variance (ANOVA) where appropriate. Groups were considered to be significantly different if $p< 0.05$. When a significant F value was obtained for ANOVA, the differences between all pairs were tested using student-Tukey-Kramer multiple comparisons test.

RESULTS

Table 1 summarizes the partial purification of the methanolic crude extract of *T. bangwensis* (mistletoe). The phytochemical screening revealed the presence of saponin, flavonoid, tannin and steroidal glycoside. The plasma glucose concentration of the experimental animals is presented in Tables 2, 3 and 4 below. The results presented in Tables 2, 3 and 4 show that treatment with methanolic crude extract of mistleoe was associated with decrease in the blood glucose concentration of the experimental animals (479.75-163.75 mg/dl) when compared with the diabetic control (232.50-377.50 mg/dl). However, the extract control group shows a mild variation of blood glucose concentration from 86.50 mg/dl to 90.00 mg/dl. This variation was however not significant as compared to the normal control group (P value is > 0.05). The extract however, shows significant reduction in blood glucose, bilubrin, cholesterol and triglycerides.

Table 1: Phytochemical components of methanolic extract of *Tapinanthus bangwensis (*Mistletoe)

4Phytochemical constituents	Results
ANTHRAQUINONE	-
ALKALOIDS	-
SAPONINS	+
FLAVONOIDS	++
TANNINS	++
STEROIDAL GLYCOSIDES	+

Key: + = positive,-= negative

Table 2: Blood glucose levels (mg/dl) of laboratory animals (rats) before inducement

No. of Cage	Group1. Normal control	Group2. Diabetic test	Group3. Diabetes control	Group4. Extract control
1	88.00	88.00	88.00	88.00
2	88.00	87.00	87.00	87.00
3	88.00	88.00	88.00	84.00
4	88.00	88.00	82.00	84.00
Mean value	88.00	87.75	86.25	85.75

Values are mean ± S.E.M (n = 4 rats per group).

Table 3: Effects of methanolic extract of *T. bangwensis* on blood glucose levels (mg/dl) on laboratory animals (rats) after 4 days of inducement with streptozocin (STZ)

No. of Cage	Group1. Normal control	Group2. Diabetic test	Group3. Diabetic control	Group4. Extract control
1	88.00	471.00	230.00	88.00
2	84.00	483.00	235.00	87.00
3	83.00	482.00	225.00	99.00
4	87.00	483.00	240.00	86.00
Mean value	85.50	479.75	232.50	90.00

Values are mean ± SEM (n = 4 rats per group)

Table 4: Effects of methanolic extract of *T. bangwensis* on blood glucose levels (mg/dl) on laboratory animals (rats) after 14 days of inducement with streptozocin (STZ)

No. of Cage	Group1. Normal control	Group2. Diabetic test	Group3. Diabetic control	Group4. Extract control
1	88.00	271.00	378.00	88.00
2	86.00	211.00	378.00	84.00
3	88.00	93.00	376.00	99.00
4	88.00	80.00	378.00	97.00
Mean value	87.50	163.75	377.50	92.00

Values are mean ± S.E.M (n = 4 rats per group)

DISCUSSION

Diabetes mellitus is a complex metabolic disease caused by impairment of insulin signaling, pathways and the defect usually results from pancreatic â-cell deficiency and/or a deficiency of insulin [4]. This disease causes many chronic complications such as vascular disease, retinopathy, neuropathy, kidney disease and heart disease. Cardiovascular disease is one of the major causes of death in diabetic patients. *Diabetes mellitus* is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk in coronary heart disease [15]. Hyperlipidemia is a recognized complication of *Diabetes mellitus* characterized by elevated levels of cholesterol, triglycerides and phospholipids and changes in lipoprotein composition [16].

This study investigated the presence of the phytochemical components and effect of *Tapinanthus bangwensis* methanolic crude extract on blood glucose level, bilirubin (total, direct and indirect), total protein and albumin levels in streptozotocin (STZ)-induced *Diabetes mellitus* (DM) in experimental animals (rats). The results showed marked changes in the characteristics of diabetic and control animals. Diabetic test and diabetic control rats showed significant ($P < 0.05$) elevated blood glucose of 479.75 ± 2.93(mg/dl) and 232.50 ± 3.23(mg/dl) Table3 after four (4) days of inducement with streptozotocin compared to 87.77 ± 0.25(mg/dl) and 86.25 ± 0.44(mg/dl) (Table2) before induction. This shows the success of induction of diabetes by streptozotocin in the experimental animals (rats). The results also show that the concentrations of blood glucose in the diabetic test (treated) group was significantly reduced to 163.75 ± 46.327 ($P < 0.05$) (mg/dl) (Table4) after fourteen [14] days of administration of aqueous *Tapinanthus bangwensis* (mistletoe) extract at 500 mg/kg body weight compared to 377.50 ± 0.50(mg/dl) (Table4) of the diabetic control (untreated) group. The report is a testimony of the efficacy of the methanolic crude extract in the control of diabetics in animal. This is supported by the report of Chauhan *et al.* [17], who reported the efficacy of the methanolic crude extract of *Centella asiatica* on the treatment of alloxan induced diabetic rats. Aqueous infusions of some medicinal plants have been reported to cause hypoglycemia in rats by increasing the level of insulin in the blood [18-20]. Thus, the observed difference in glucose concentrations indicates probable hypoglycemic properties of the aqueous extract of *Tapinanthus bangwensis* on streptozotocin induced diabetic rats. Therefore, it is obvious that the fractionation with methanol has enriched the active phytochemical components. In glucose loaded animals, the active components have reduced the blood glucose level to the normal. It is possible that, the active components act by potentiating the pancreatic secretions or increasing the glucose uptake.

Hypercholesterolemia, hypertriglyceridemia and hyperurea have been reported to occur in drugs (alloxan) induced diabetic rats [20, 21] and a significant increased observed in our experiments with streptozotocin (STZ) induced diabetic rats was in accordance to these studies.

In conclusion, the aqueous extracts of *Tapinanthus bangwensis* (mistletoe) leaves exhibited hypoglycemic property for use as anti-diabetic herb, but do not induce adverse alterations in biochemical parameters such as

serum total protein, albumin and bilirubin. Aqueous extract of mistletoe leaves may serve as a good candidate for alternative and/or complimentary medicine in the management of diabetes. Researches should be intensifed to isolate the active components from menanolic extract of *T. bangwensis* and elucidate the mechanism of action.

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