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Effect of Aqueous Extract of *Tapinanthus bangwensis* on the Hyperglycaemic Levels of Alloxan-Induced Diabetic Wistar Rats

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ABSTRACT

This study investigated the effect of aqueous extract of *Tapinanthus bangwensis* on the hyperglycaemic levels of alloxan-induced diabetic Wistar rats. The effect of the plant extract was monitored on the serum concentration of glucose (GLU). Sixty-six Wistar rats were used for the study arranged into eleven groups of six rats each. Two groups (12 rats) were used for pilot study. 9 other groups (54 rats) were used for experiment labelled groups 1-9. Group 1 constitute the normal control which received only feed and water, group 2 received 50mg/kg citrate buffer. Group 3 was administered alloxan solution and allowed free access to feed and water. Group 4-6 received 50mg/kg of citrate buffer and were also administered with the aqueous extract of *Tapinanthus bangwensis* at a dose of 250mg/kg and referred to as normal treated, concentration 1(NT conc-1), concentration 2 (NT con-2) and concentration 3 (NT conc-3) respectively. Group 7-9 were administered 50mg/kg alloxan and different grades (5%, 7%, 10%) respectively of the aqueous extract. Blood, liver and pancreatic tissue samples were collected into appropriately labelled sample bottles and analysed. Result of the blood analysis showed that the serum glucose levels were significantly elevated ($p<0.05$) in groups 3 and 6-9. The serum level increased to 7.85±0.69 compared to the 2.47±0.27 control value. Oral administration of 250mg/kg aqueous mistletoe extract to groups 7,8,9 of 50mg/kg 70mg/kg and 100mg/kg respectively significantly($p<0.05$) decreased some of those biochemical alterations in a dose-dependent manner. 100mg/kg administration of the extract showed the highest effect in lowering the elevated parameters followed by 70mg/kg administration. 50mg/kg had the least lowering effect. Glucose was lowered from 7.85±0.69 to 6.92±0.64, 6.07±0.22, 5.30±0.30 in group 7,8 and 9 respectively. Hence, *Tapinanthus bangwensis* has hyperglycaemic potentials.

Keywords: *Tapinanthus bangwensis*, Hyperglycaemic Levels, Aqueous extract, highest effect, Diabetic Rats

I. INTRODUCTION

Man has continued to launch an endless search for substances or agents with medicinal value that could help him confront the numerous health challenges resulting from environmental, metabolic and pathogenic problems while bearing in mind the economic implication of these medicinal agents [1,2]. In the resent timed, this search has gathered much momentum towards plants and plant products because of their little or no side effects and their rich medicinal values [3,4].

Natural products and their derivatives represent more than 50% of all drugs in clinical use in the world. Higher plants contribute about 25% of the total drugs from natural sources [5,6].

Odia and Wokoma and recently Omeodu *et al*. have stated that diabetes mellitus is the major cause of morbidity and mortality globally [7,8]. Between 1980 and 2004 alone diabetic Americans rose from 5.8-14.7 million with 1.4 million cases recorded in 2004, within the ages of 18-79 years old. The number is expected to increase to 29 million in 2005 [9]. The disease diabetes mellitus is distinct from another type of diabetes called diabetes insipidus which is a rare metabolic disorder (the incidence of diabetes insipidus is 3 in 100,000 persons characterised by the production of large volume of urine by the patient [10].

Diabetes mellitus simply referred to as diabetes has been defines by World Health Organization (WHO) as fasting venous plasma glucose concentration greater than 7.8mol/l (140mg/dl) or greater than 11.1mmol/l (200mg/dl) 2 hours after a carbohydrate meal or 2hours after an oral ingestion of the equivalent of 75g glucose even if the fasting concentration is normal [11].

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The purpose of this research is to investigate if aqueous extract of *Tapinanthus bangwensis* has an effect on the hyperglycaemic levels of diabetic Wister rats after alloxan-inducement and if the plant can be used for management of diabetes through the reduction of some of the several disordered biochemical parameters.

II. MATERIALS AND METHODS

Preparation of Plant materials

The plant used for this work is *Tapinathus bangwensis*. The plant was found in the University of Port Harcourt where it was found hemi-parasitizing on specie of orange (*Citrus aurantium*) 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 24 days until a constant weight was obtained.

Preparation of aqueous extract of *Tapinanthus bangwensis*

Tapinathus bangwensis was collected with stalks. The fresh greenish leaves were carefully plucked off from the stalks and pedicle removed from each leaf. The leaves were thoroughly washed and spread out on a clean cardboard paper and kept at room temperature in a well aerated room. They were allowed to dry to constant weight after 24days. The dried sample was then pounded in a mortar with pistil. After pounding, the partially powdered sample was grounded in a manual grinding machine until a fine powder was obtained. Fifty grams of the powdered mistletoe was measured and dissolved in a 1 litre measuring cylinder containing 500ml distilled water. The mixture was thoroughly shaken for 10 minutes. The mixture was then stored at room temperature for twenty-four hours [12]. The preparation was filtered using ten different pieces of white cloth. The filtrate was filtered two times through a Whitman No. 541 filter paper and stock was stored in a refrigerator at a temperature of 40^{o}C for 24 hours. 50mg/kg, 70mg/kg and 100mg/kg of the filtrate were then prepared from the stock solution and these three different concentrations were used to treat the test animals [12].

Experimental animals

The animals were divided into experimental groups of six (6) animals per group and each group was housed in a metallic cage. They were provided with feeds and water ad libitum. The animal feeds were purchased from the Livestock Feeds, Choba, a division of Livestock Feeds Nigeria Limited, Ikeja, Lagos; while the water was supplied by the Water Treatment Plant, Choba Park, University of Port Hracourt. There was a total of (9) experimental group. All the rats weighed between 200g-300g and their average age was fourteen (14) months.

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

Table 1: Research Design

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**	**Group 9**
	Normal control 1 (NC-1)	Normal control 1 (NC-2)	Normal diabetic control (NDC)	Normal treated control (NT-1)	Normal treated control (NT-2)	Normal treated control (NT-3)	Diabetic treated control (DT-1)	Diabetic treated control 2 (DT-2)	Diabetic treated control 3 (DT-3)
No of Rats	6	6	6	6	6	6	6	6	6
Treatment	Feed + H_2O Only	Feed + H_2O *ad libitum* +	Feed + H_2O *ad libitum*	Feed + H_2O	Feed + H_2O *ad*	Feed + H_2O *ad libitum* +	feed + water *ad*	Feed + water *ad libitum*	Feed + water *ad*

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	ad libitum	citrate buffer	+ alloxan solution	*ad libitum* + citrate buffer 5% mistletoe solution	*libitum* + citrate buffer + 7% mistletoe solution	citrate buffer 10% mistletoe solution	*libitum* + alloxan + 5% mistletoe solution	+alloxan + 7% mistletoe solution	*libitum*+ alloxan solution + 10% mistletoe solution.

Group one animals were administered only feeds and water *ad libitim* to serve as general control group. Group two animals received citrate buffer solution in addition to feeds and water. Alloxan solution was administered to group three animals and allowed free access to feed and water. Before citrate and alloxan administration to group two and three respectively, the animals were fasted for 18 hours. This was the same for group 4 to 9 animals that received various treatments. Group four to six were administered with citrate buffer at 50mg/kg dose, while groups seven to nine were administered with alloxan solution at same 50mg/kg and then treated with *Tapinanthus bangwensis* solution at a dose of 250mg/kg with group seven receiving 50mg/kg of the *Tapinanthus bangwensis* extract, group eight receiving 70mg/kg and group nine 100mg/kg of the extract.

Administration of *Tapinanthus bangwensis* extract

The *Tapinanthus bangwensis* solution was prepared into 5%, 7% and 10% by the process already stated by Omeodu *et al*. [12]. These three different preparations were fed only to groups 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 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 the cage and sacrificed by cervical dislocation [13]. The blood sample was then collected from the animal after withdrawing from the chamber by cardiac puncture into appropriately labelled sample bottles. These samples at the end of collection were quickly taken to the laboratory for analyses. The blood specimen was centrifuged at 5000rpm using MSE centrifuge to obtain plasma.

Glucose assay

Test principle. Serum glucose of the rest animals were estimated using the glucose oxidase method [14]. Glucose is oxidized to gluconic acid and hydrogen peroxide. The hydrogen peroxide was acted upon by peroxidase in the presence of 4-aminophenazone to form a red violet quinoneimine dye as an indicator which was measured spectrophotometrically at 520nm wavelength.

III. RESULTS

The results of the investigation shown on figure 1 indicated clearly that alloxan caused diabetes mellitus in the experimental animals. Values of the control animals were found to be within normal range for the parameters analysed. Figure 1 illustrates the effect of aqueous extract of mistletoe on alloxan-induced diabetic rats and its effect on serum glucose level. Glucose level was markedly increased in alloxan diabetes as seen in group 3 but was lowered on a dose dependent manner when the rats were treated with mistletoe extract.

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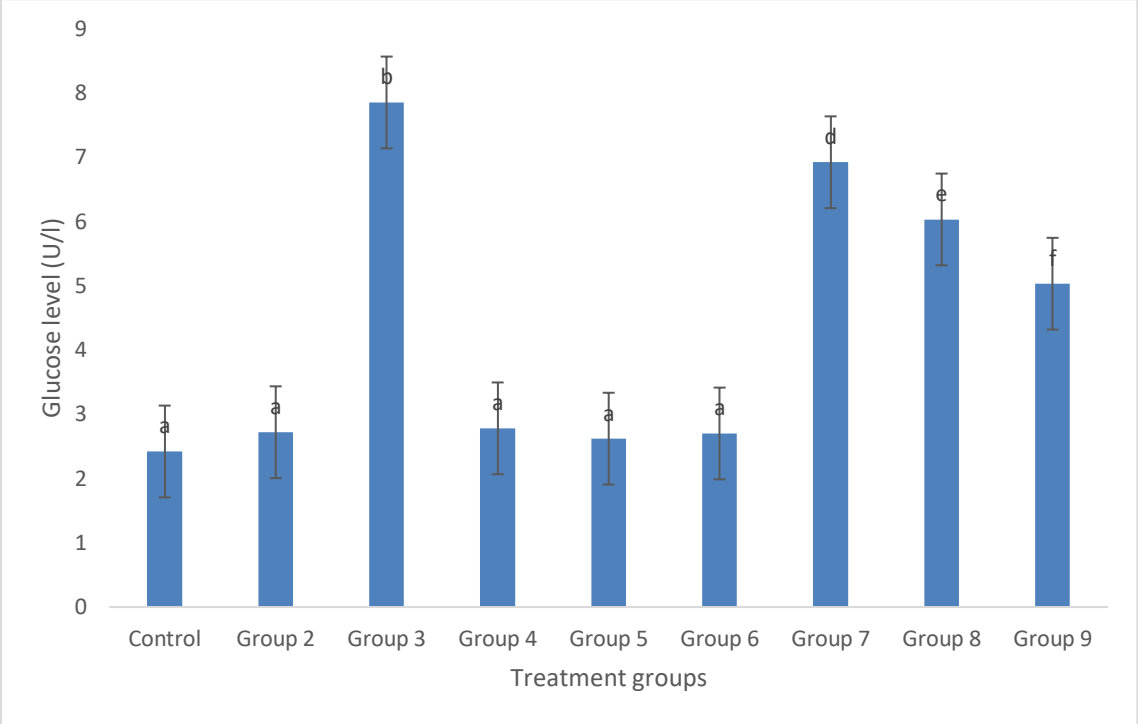

Figure 1: Serum glucose concentration in alloxan-induced diabetic rats treated with mistletoe extracts. Values are expressed as Mean ± Standard error of mean (SEM), n=5. Values with the same are not significantly different at ($p<0.05$).

IV. 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 [15]. Swartson-Flatt *et al*. reported that many clinical parameters associated with diabetes mellitus in experimental animals were reduced when administered with the extract of mistletoe (6.25%) by weight [16]. He observed that such diabetes associated symptoms as polydipsia, polyphagia and body weight loss were all ameliorated with mistletoe administration. The work of Diden *et al*. has lent credence to this present work [17].

In this work, it was observed that the alloxan given to the rats at a dose of 50mg/kg induced diabetes mellitus in them. Diabetes was not observed when the carrier solvent, citrate buffer was administered to non-diabetic rats; hence the diabetogenicity was caused by only alloxan [18]. This resulted to a significant ($p<0.05$) increase in blood glucose level as seen in figure 1.

Treatment of test animals in group 3 with alloxan significantly ($p<0.05$) elevated their serum glucose compared with the control. Treatment of animals with citrate buffer alone in group 2 did not cause any significant serum elevation of glucose when compared with the control. Pari and Amarnath suggested a possible mechanism for serum glucose reduction by herbal extracts as potentiation insulin release from the beta cells of the pancreas [19]. The result of this study agrees with the works of Aina *et al*. [20]. Akah and Okafor results were also in agreement with the result of this work [21].

The observed serum glucose increases for the diabetic treated groups suggest that *Tapinanthus bangwensis* extract is insulinogenic hence stimulates insulin secretion from remnant beta cells. Hyperglycemia generates abnormally high levels of free radicals by autioxidation of glucose and protein glycation, and oxidative stress have been reported to be a causative factor of cardiovascular complications in alloxan-diabetes mellitus [22]. Hyperglycemia is associated with the generation of reactive oxygen species (ROS) causing oxidative damage particularly to the heart, kidney, eyes, nerves, liver, small and large vessels and gastrointestinal system [18,23].

The increased level of plasma glucose was lowered by administration of aqueous *Tapinantus bangwensis*. Extracts and this effect have been attributed to the potentiation of insulin from existing β-cells of the islets Langerhans. The

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mechanism involved in the action may be similar to that of a standard therapeutic-drug like glibenclamide which can stimulate insulin secretion from pancreatic β-cells [24]. However herbal drugs have little or no side effects [25,26].

V. CONCLUSION

This study was able to establish the diabetogenicity of alloxan as seen in the glucose level, and that in diabetes mellitus, glucose concentrations are elevated. Therefore, this research has shown that extracts of African *Tapinanthus bangwensisis* 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 mellius.

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