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Hypoglycemic Effect of Methanolic Extract of *Anacardium occidentale* leaves in Alloxan-Induced Diabetic Rats

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Summary: *Anacardium occidentale* Leave (Anacardiaceae), a plant natively grown in wastelands in Africa is used as a folk remedy for diabetes mellitus. Previous studies, reported the hypoglycemic effect of the aqueous leaf extract of A. occidentale in diabetic rats and its prophylactic activity against the diabetogenic action of streptozotocin This study evaluated the hypoglycemic effect of a methanolic extract of streptozotocin leaves and its fractions in Alloxan-induced diabetic rats in comparison to Tolbutamide, a reference drug. For moderately diabetic rat, A. occidentale caused a 79.2 % change over 4 hours and Tolbutamide caused a 63.1 % change over this same time period. When the rat were considered to be severely diabetic, the A. occidentale decreased the blood glucose levels by 20.8% change over four hours and the mean percent change over 4 hours for Tolbutamide was 47.63 %. These values were not considered significant ($p>0.05$). So the same conclusion can be made about the efficacy of A. occidentale, when compared to the reference drug, Tolbutamide. These results that show that A. occidentale has a similar ability compared with Tolbutamide to lower blood glucose levels.

Keywords; *Anacardium Occidentale*, Tolbutamide., Hypoglycaemia, Alloxan-Induced Diabetes
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INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macro vascular complications that result in a significant morbidity and mortality. The increasing proportion of the aging population, consumption of calorie rich diet, obesity and sedentary lifestyle have led to tremendous increase in the number of diabetics worldwide (George, 2000). Although, providing good glycemic control, current therapies do little in preventing complications. Besides this, these drugs are associated with side effects. Thus, it is necessary to continue looking for new and if possible more efficacious drugs. Diabetes mellitus (DM) is a chronic metabolic condition characterized by disorder of glucose homeostasis. Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to excess formation of free radicals.

The traditional treatments of diabetes may include a low sugar and carbohydrate diet accompanied with exercise for mild cases. For more severe and harder to control glucose levels, diabetes can be treated by the administration of insulin and/or by sulfonylurea antidiabetic drugs that stimulate the production of insulin and therefore lower the blood glucose levels. The reference drug that will be used in this experiment is tolbutamide, which starts to work within one hour and is effective for 6-12 hours. For sulfonylurea antidiabetic drugs to work there has to be some cells of the pancreas that are working and therefore are able to be stimulated to produce appropriate amounts of insulin (Silverman, 1986).

Tolbutamide, along with all the other sulfonylureas drugs, increases the output of insulin by binding to the receptors of the beta cells of the Islets of Langerhans located in the pancreas. Once they bind to the sulfonylureas receptors, the K+ -ATP channels are closed and therefore the membrane is depolarized and insulin production is stimulated (Rorsman, 1997).

More than 400 species of plants have been reported to display hypoglycemic effects, but only few of them have been investigated (Miura et al 2002)and the World Health Organization has recommended that much research should be done on hypoglemic plants (WHO, 2002)

In this study, our attention focussed on hypoglycemic effect of a methanolic extract of *A occidentale* leaves and its fractions in alloxan-induced diabetic rats. Previous studies, reported the hypoglycemic effect of the aqueous leaf extract of *A. occidentale* in diabetic rats and its prophylactic activity against the diabetogenic action of streptozotocin [Kamtchouing, 1998. Paris, R., 1977) *Anacardium occidentale* L. (Anacardiaceae) is, a plant natively grown in wastelands in Africa and is used as a folk remedy for diabetes mellitus [Longuefosse, 1996)]. The plant is an ornamental tree up to 10 m high and is widely distributed in tropical countries (Paris, 1977).

This study is going to evaluate the hypoglycemic effect of a methanolic extract of *A occidentale* leaves and its fractions in alloxan-induced diabetic rats. Also, it will look at how effective these treatments are in lowering glucose levels over a short period of time (2-4 hrs). The ability to decrease hyperglycemia in the rat by the medicinal plant treatment will be compared to that of Tolbutamide, a reference drug
.

MATERIALS AND METHODS

Plant material collection

The leaves of *A. occidentale* were collected from Obasanya Compound Odo-Osun Street Ijero Ekiti, Ekiti State Nigeria and identified by Dr Asidi S of botany department Olabisi Onabanjo University,Ago-Iwoye,Ogun State,Nigeria.

Preparation and Fractionation of the Methanol Extract:

The Leaves washed in tap water and dried at room temperature. The dried leaves were powdered mechanically and sieved using a fine muslin cloth. The fine powdered leaves were kept separately in airtight containers in a room temperature until the time of use.

The methanol extract was prepared by mixing 400 g of plant powder with 1500 ml methanol for 48 h three times at room temperature and filtering through No. 3 Whatman filter paper at room temperature. The combined extracts were evaporated under reduced pressure using a rotary evaporator to obtain 54.5 g of dried material yielding 13.70%. The dried sample was suspended in water and then partitioned successively with n-hexane, dichloromethane and ethyl acetate. The parent methanol extract and its fractions were tested in diabetic rats.

Experimental animals

Male albino Wistar rats (150 to 200 g) bred in the Vertinary Physiology and Animal house,university of Ibadan, Nigeria, were used in this study. The animals were fed on a pellet diet (Hindustan Lever, India) and water *ad libitum.*

The animals were maintained in their respective groups for 60 days. All studies were conducted in Biochemistry Laboratory. Olabisi Onabanjo University Ogun State.

Alloxan-induced Hyperglycemia

Diabetes was induced in male Wistar albino rats by intraperitoneal administration of alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline given at 48-hour intervals. The rats were fasted for 18 hours before the first injection.

Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 30 percent glucose solution orally at different time intervals after six hours of alloxan induction, and 5 percent glucose solution was kept in bottles in their cages for the next 24 hr to prevent hypoglycemia.

After 7 days, rats with diabetes mellitus which have glycosuria (indicated by Benedict's test) and hyperglycemia with blood glucose range of 250 to 375 mg/dl were used for the experiment.Induction of diabetes was accomplished by three doses of alloxan that was administered at different time interval. One week after the third injection, the rats were fasted again and the blood glucose levels were recorded.

Experimental design: Animals were divided into eight groups of six rats each. Feed and water were provided to the animals:

- Group 1, Control vehicle only (2 percent gum acacia).
- Group 2, Severely Induced Diabetic Rat + methanolic extract of *A occidentale* leaves (>300 mg/dl of blood glucose)
- Group 3, moderately Induced Diabetic Rat+ methanolic extract of *A occidentale* leaves (100 and 300 mg/dl of blood glucose)

- Group 4, Severely Induced Diabetic Rat + Tolubutamide
- Group 5 moderately Induced Diabetic Rat +Tolubutamide
- Group 6 Severely Induced Diabetic Rat + Tolubutamide + methanolic extract of *A occidentale* leaves
- Group 7 moderately Induced Diabetic Rat +Tolubutamide + methanolic extract of *A occidentale* leaves

Biological Assays: The hypoglycemic activity of the plant extracts and its functions were evaluated in acute and sub chronic experiments. In the experiment, blood samples were taken for the glucose determination from overnight fasting rats, and afterwards, methanol extract plant and tolubutamide were administered respectively by gastric intubation to four groups of five diabetic rats each at respective doses of 200, and 400mg/kg. The dried methanol extract was dissolved in a 1% dimethyl sulfoxide (DMSO) water solution (v/v) before administration.

Control animals were given vehicle (1% DMSO water solution, v/v). The blood sample was obtained by using the tail clip method (Alarcon-Aguilar et al, 2000) 2, 4hrs after the injection from rat tail tip. A drop of blood was placed on a blood glucose test strip and was inserted into a glucometer. Rats were categorized as either mildly or severely diabetic according to their blood glucose levels. Rat with basal blood glucose levels between 100 and 300 mg/dl were considered moderately diabetic and rats with basal glucose levels over 300 mg/dl were labelled as severely diabetic. The percentage change in blood glucose was calculated by applying the following formula [Hamden and Afifi, 2004]: % Change of Glycemia = [(Ax-A0)/A0] x 100

The percent change of the glucose levels were measured for the two and four and six hour time period for each treatment (before-after/before x 100). A T-test for the means of percent change for both time periods and the two treatments were compared according to this data.

Blood sampling and Biochemical Estimations: Blood samples (1.0 ml) for glucose determination were obtained from the tail tip. Blood glucose level was measured with an ACCUTREND GC blood glucose analyser (Boerhinger Mannheim, Germany).

Statistical analysis: Values were represented as mean ± SD. Data was analyzed using analysis of variance and group means were compared with Duncan's multiple range test.

RESULTS

Table 1 shows the blood glucose levels before treatment, two hours after, four hours and six hours after treatments with Tolbutamide and *A. occidentale*, and between severity of diabetes.

Table 2 shows the average percent changes of blood glucose levels that were seen at the 2 hour and 4 hour time period for *A. occidentale* and Tolbutamide in both the moderately and severely diabetic rat. In Table 2, the p-value from the T-test that compared the mean percent change of the two different treatments is also represented. The p-values for moderately diabetic rat after two and four hours showed that the means were not significantly different ($p>0.05$) for *A. occidentale* and Tolbutamide. The same result was seen in the severely diabetic rat when comparing the mean percent change after 2 hours,4 hours respectively , the p-value (>0.05) and so the means were not significantly different.

Table 1;
Comparison of Average Glucose Levels (mg/dl) in Moderately and Severely Diabetic Rats Comparison

Treatment	Initial	2hrs PT	**4hrs PT**	**6hrs PT**
Anacardium occidentale L (moderate)	452 ±5.3	359 ±5.8	331±4.5	317 ±6.3
TLBT (moderate)	452 ±4.8	327 ±4.3	238±3.6	196 ±3.5
Anacardium occidentale (severe)	256 ±5.3	158 ±5.6	57 ±0.3	48 ±0.8
TLBT (Severe)	190.6 ±3.8	97 ±0.4	65.7 ±0.3	56 ±0.3
Anacardium occidentale + TLBT L(moderate)	452 ±4.3	235 ±4.5	208 ±7.5	198 ±4.5
Anacardium occidentale + TLBT (severe)	256 ±5.0	110 ±3.5	101.5 ±4.9	46 ±0.9

TLBT = Tolubutamide; PT = Post treatment

DISCUSSION

Blood sugar level increased as expected in alloxan-injected animals, since alloxan causes a massive reduction in insulin release, by the destruction of the

beta-cells of the islets of Langerhans and inducing hyperglycemia

For moderately diabetic rat, *A. occidentale* caused a 79.2 % change over 4 hours and Tolbutamide caused a 63.1 % change over this same time period. Since the p value ($p>0.05$) showed there was no significant difference between the mean percent change for the two treatments, the conclusion can be made that *A. occidentale* has a very similar ability to lower glucose levels in moderately diabetic rat.

Table 2:
Comparison of mean% change in average blood glucose in moderate and severely diabetic Rats

Treatment	Mean % change after 2hrs	Mean% change after 4hrs	p-value
Anacardium occidentale L(moderate)	39.75±0.1	79.2±2.0	p>0.05
Tolubutamide (moderate)	40.4±0.01	63.1±2.0	p>0.05
Anarcardium occidentale (severe)	20.4±2.5	20.8±3.2	P>0.05
Tolubutamide (Severe)	27.1±2.4	47.6±7.0	p>0.05
Anacardium occidentale +Tolubutamide L(moderate)	36.3±2.1	85.4±7.0	p>0.05
Anarcardium occidentale + *Tolubutamide (severe)*	22.8±2.1	51.6±3.0	p>0.05

When the rat were considered to be severely diabetic, the *A. occidentale* decreased the blood glucose levels by 20.8% change over four hours and the mean percent change over 4 hours for Tolbutamide was 47.63 %. These values were not considered significant ($p>0.05$).*A.occidentale* has effectevely reduced blood glucose and that its effect is similar to that of the reference drug. So the same conclusion can be made about the efficacy of *A. occidentale*, when compared to the reference drug(Tolbutamide). The blood glucose levels achieved after the four hour time period for the severely diabetic rat are still considered to be in the diabetic range, so further research needs to be done to find the optimal dose for severely diabetic people.

These results that show that *A. occidentale* has a very similar ability to Tolbutamide, a reference drug, to lower blood glucose levels, one can conclude that it can be considered as an alternative treatment for people who are suffering from diabetes. Further research needs to be done to fully understand how *A. occidentale* is working within the pancreas and by what mechanism it is able to lower glucose levels in alloxan-induced diabetic rat.

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