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Research Article

EVALUATION OF THE ANTI DIABETIC ACTIVITY OF ETHANOL EXTRACT OF *ANCHOMANES DIFFORMIS* (ARACEAE) LEAVES IN ALBINO RATS

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ABSTRACT

The leaves of *Anchomanes difformis* are used in traditional medicine to promote health in the ageing. This study investigated the antidiabetic activity of the ethanol extract of the leaves of *Anchomanes difformis* in albino rats. Diabetes was induced by intra-peritoneal injection of 160 mg/kg of alloxan monohydrate. Plant extract (250 mg/kg and 500 mg/kg) was administered orally and blood glucose levels were monitored at 2 hourly intervals and daily for 14 days. The LD_{50} was found to be greater than 5000 mg/kg. Phytochemical screening showed the presence of tannins, phlobatannins and alkaloids, flavonoids, reducing sugars, saponins and cardiac glycosides. The two doses of the extract produced a statistically significant reduction of fasting blood glucose levels during the acute study and prolonged treatment for 14 days when compared to glibenclamide and the negative controls. The plant extract seems to possess a dose-dependent anti diabetic effect. Further studies will be done to evaluate the toxicological effects of this plant extract.

Keywords: Anti diabetic, *Anchomanes difformis*, alloxan, ethanol, Phytochemical

INTRODUCTION

Diabetes Mellitus is a chronic non–communicable disease. The World Health Organization (WHO) predicted that the global prevalence of diabetes mellitus would rise from 2.8 % (171 million) to 4.4 % (366 million) by 2030, with the most significant increase predicted to occur in developing countries. Diabetes mellitus is a chronic progressive metabolic disorder characterized by hyperglycaemia mainly due to absolute (Type 1 DM) or relative (Type 2 DM) deficiency of insulin. Diabetes mellitus virtually affects every system of the body mainly due to metabolic disturbances caused by hyperglycaemia, especially if diabetes control over a period of time proves to be sub-optimal[1]. Diseases such as cardiovascular disease, Alzheimer's disease, Parkinson's disease, diabetes, cancer, arthritis and other inflammatory conditions as well as ageing are associated with oxidative stress[2]. Hyperglycaemia in diabetes is associated with a higher risk of retinopathy, macro vascular disease, increased carotid media thickness, oxidative stress, inflammation, endothelial dysfunction, some cancers and impaired cognitive function[3]. The associated complications of synthetic drugs have led to a shift towards locating natural resources showing anti-diabetic activity. The Indian prehistoric literature reports more than 800 plants with anti-diabetic properties while ethno-pharmacological surveys indicate that more than 1200 plants can be used for hypoglycaemic activity[4]. *Anchomanes difformis*is commonly used in traditional medicine to treat many diseases[5,6] whose pathogenesis are, among other factors, linked to oxidative stress. *Anchomanes difformis* is a plant in the family of Araceae and is common in West African forests. The local Nigerian names are abirisoko (Yoruba), Olumahi (Igbo) Eba-enang (Efik). Herbs are known to provide symptomatic relief and aid in the prevention of the secondary complications of the disease including cholesterol lowering action. Some of these herbs have also been proven to help in the regeneration of β-cells and in overcoming insulin resistance. In addition to maintaining normal blood sugar level, many of these plants possess antioxidant activity[7]. It has been reported by[8] that the root extracts of *Anchomanes difformis* possessed strong concentration-dependent free radical scavenging and antioxidant activities. The aim of this study was to determine the anti diabetic effect of the ethanol extract of the leaves of *Anchomanes difformis* on alloxan induced diabetes in albino rats.As far as we know; this plant has not previously been evaluated for its potential antidiabetic activity.

MATERIALS AND METHODS

Plant Collection and Identification

The leaves of *Anchomanes difformis* were collected from Rumuoparaeli forest, Choba, Rivers state, Nigeria and was authenticated by D.E. Essemenochai of the Department of Botany, University of Ibadan, with herbarium number UIH-22361. A voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy and Phytotherapy, University of Port Harcourt.

Preparation of Plant Extract

The leaves of *Anchomanes difformis* was dried under a shade for one week and pulverized into fine powder in an electric blender. The plant powder was subjected to solvent extraction by maceration for 72 h using absolute ethanol (Sigma, Aldrich). The filtrate was concentrated with a Rotary evaporator at 60^{o}C. The extract was further evaporated to dryness over a water bath set at 40^{o}C. The percentage yield was then determined and the extract was stored in a refrigerator.

Experimental Animals

Albino rats were procured from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Port Harcourt and Rivers State. The animals were housed in separate cages and had free access to feed and water *ad libitum*. They were allowed to acclimatise for two weeks. All the animals were fed with rodent pelletized feeds (manufactured by

Pfizer, Nigeria and distributed by Golden penny premier feed mills company Limited) under strict hygienic conditions.

Determination of Acute Toxicity

This was carried out according to[9] method. Eighteen albino rats of both sexes with average weight of 200 g were used in this study. The animals were divided into six groups of three animals each. The animals in the first three groups were treated with 10 mg/kg, 100 mg/kg and 1000 mg/kg of the extract. They were kept under observation for 24 h for signs of toxicity or death. None of the animals died. The remaining three groups were then administered with the extract at doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg, respectively. The animals were again observed for 24 h.

Experimental Procedure

The method of[10] was modified and adopted. Animals of both sexes were used for this study. Five animals were selected and treated as normal control group as diabetes was not induced in these animals. Other animals were fasted overnight and diabetes was induced by intra-peritoneal injection of 160 mg/kg of a freshly prepared solution of alloxan monohydrate (Aldrich Chemistry, a division of Sigma Aldrich Company, U.S.A.) After 72 h, the animals with blood glucose levels above 200 mg/dl (diabetic) were selected for the study[11]. The animals were randomly divided into five groups of five rats each. Groups I and II were administered with 250 mg/kg and 500 mg/kg of the extract, group III was administered with glibenclamide, group IV was administered with distilled water while group V was the normal control group mentioned earlier. The changes in body weight and fasting blood glucose level of all the rats were monitored and recorded at regular intervals during the entire study period. Fasting blood glucose level was determined on day 0, 1, 3, 5, 7, 10 and 14, every morning, before food administration, following an overnight fast. Blood glucose levels were determined for the acute study at 0 h, 2 h, 4 h and 6 h on day 0. The blood glucose levels were monitored in the blood of the diabetic rats by tail snipping method. The blood was dropped on the Accu-check active blood glucose meter (Roche Diagnostics, Germany) reagent test strip. This was inserted into micro processor digital blood glucometer and the readings were recorded in mg/dl[10].

Statistical Analysis

Data are expressed as mean ± SEM. Statistical analysis of data was done using Microsoft excel. Student's t-test was done to determine the significance of difference between the control groups and the treated groups. P-values < 0.05 were considered to be statistically significant.

Table 1: Effect of ethanol extract of *A. difformis* leaves on body weights of alloxan-induced diabetic rats (Mean ± S.E.M)

Treatments	Dose mg/kg	Body Weights		% Increase/ Decrease in Body Weights
		Day 0	Day 15	
Extract	250	200.2 ± 11.30	169.6 ± 13.21	-15
Extract	500	214.2 ± 19.66	190.8 ± 15.25	-11
Glibenclamide	10	226.6 ±17.83	158 ± 4.55	-30
Diabetic Untreated	2 ml	246.3 ± 9.69	209.8 ± 17.67	-14.8
Normal Control	2 ml	218.6 ± 16.96	227 ± 12.80	3.84

Table 2: Anti diabetic effect of ethanol extract of *A. difformis* on blood glucose level of alloxan-induced diabetic rats during acute study (Mean ± S.E.M)

Treatments	Dose (mg/kg)	Blood Glucose Level (mg/dl) in Hours			
		0	2	4	6
Extract	250	443 + 24.3*	288.2 + 67.53*	321.8 + 18.24*	315 + 17.08*
	500	380.8 + 38.84*	294.8 + 43.21*	378.4 + 37.70*	381 + 20.70*
Glibenclamide	10	531.20 + 36.21	447.00 + 54.62	478.00 + 53.96	485.8 + 39.33
Diabetic Untreated	2 ml	558.60 + 24.24	485.60 + 50.19	465.60 + 57.78	511.4 + 13.67
Normal Control	2 ml	86.60 + 6.02	99.20 + 3.02	106.00 + 2.82	107.2 + 4.99

*= P < 0.05 compared to the diabetic untreated group

Table 3: Effect of ethanol extract of *A. difformis* on blood glucose level of alloxan-induced diabetic rats during prolonged treatment (Mean ± S.E.M)

Treatments		Extract		Glibenclamide	Diabetic Untreated	Normal Control
		250 (mg/kg)	500 (mg/kg)	10 mg/kg		
Blood glucose level (mg/dl) in days	Day 0	443 ± 24.3	380.8 ± 38.84	531.20 ± 36.21	558.60 ± 24.24	86.60 ± 6.02
	Day 1	325.20 ± 39.04*	389.60 ± 58.29*	389.60 ± 58.29*	591.60 ± 1.86	121.40 ± 3.12
	Day 3	357.40 ± 61.45*	127.20 ± 41.79*	317.00 ± 70.90*	594.40 ± 1.07	137.40 ± 15.01
	Day 5	197.80 ± 83.43*	191.40 ± 33.79*	269.80 ± 74.17*	580.60 ± 3.47	119.00 ± 8.93
	Day 7	425.00 ± 65.42*	201.60 ± 34.86*	346.60 ± 47.90*	590.40 ± 2.99	125.20 ± 10.36
	Day 10	309.00 ± 13.98*	270.80 ± 55.23*	214.80 ± 83.91*	585.60 ± 3.58	116.40 ± 11.70
	Day 14	389.60 ± 38.56	233.40 ± 48.22*	273.60 ± 55.16	363.20 ± 14.94	109.40 ± 10.97

*= P < 0. 05 compared to the diabetic untreated group

RESULTS

There were observable changes in the body weight of both the treated and untreated diabetic rats. Treatment of rats with the extract of *Anchomanes difformis* was associated with weight loss.A decrease in body weight was observed in rats treated with glibenclamide, 250 mg/kg and 500 mg/kg of the extract while there was an increase in the normal control group (Table 1). It was observed that the blood glucose levels of the extract treated groups and the glibenclamide treated groups were fluctuating during the duration of the acute study. A decrease in blood glucose levels was observed in the extract treated and glibenclamide treated groups at 2 h. However, the blood glucose levels in these groups were increased at 3 h. A further decrease in blood glucose level was observed in the group that was administered with 250 mg/kg of the extract. However, the extracts seemed not to possess anti diabetic effect during the acute study (Table 2). During the prolonged treatment study, a gradual reduction in the blood glucose levels of the extract treated and the glibenclamide treated groups from day 0 to day 5. Meanwhile, there was an increase in the blood glucose levels on day 7 which further showed a decrease on day 10 and day 14. However, there was a statistically significant difference in the blood glucose level from day 1 to day 10 when compared to the diabetic untreated. The 500 mg/kg of the extract group showed a statistically significant ($P < 0.05$) decrease in blood glucose levels until day 14. The plant extract exhibited a dose-dependent antidiabetic activity (Table 3).

DISCUSSION

Preliminary phytochemical screening of the ethanol extract of *Anchomanes difformis* leaves revealed the presence of tannins, phlobatannins and flavonoids, reducing sugars, saponins and cardiac glycosides. The LD_{50} was found to be greater than 5000 mg/kg and could be considered as relatively safe[9]. The decrease in body weights of the treated groups and the diabetic untreated is one of the conditions that characterizes diabetes which may be due to loss or degradation of structural proteins[12]. The plant extract did not show anti diabetic effect during the acute study while it exhibited a dose–dependent statistically significant anti-diabetic activity within the period of both acute and prolonged treatment when compared to the negative control group. Alloxan has been reported to cause a massive reduction in insulin release by the destruction of β-cells of the islets of Langerhans, thereby inducing hyperglycaemia[13]. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage. During normal metabolism and energy production in the body, oxygen derived free radicals or Reactive Oxygen Species (ROS) as well as Reactive Nitrogen Species (RNS) are generated[14]. They are produced to help the normal healthy tissues perform physiological roles such as signalling molecules, regulation of signal transduction and gene expression, activation of receptor and nuclear transduction among others [15]. But when these ROS or RNS are present in higher concentration beyond the antioxidant capacity of a biological system, due to metabolic and other environmental factors; it gives rise to an imbalance known as oxidative or nitrosative stress[8]. In this study, continuous treatment with the extract for a period of two weeks caused significant decrease in the blood glucose level of treated rats compared to untreated diabetic rats. The finding of this study corroborates the report of[16] on *Musa sapientum* Kuntze (Banana) which significantly showed anti hyperglycaemic action and an antioxidant effect. Banana flower was more effective than glibenclamide. Some phytochemical compounds in plants such as saponins, flavonoids and triterpenes have been reported to be responsible for the anti diabetic activities of plants[17]. Also,[18] reported that the stem extracts of *Euphorbia hirta* has significant anti hyperglycaemic effects which may be due to antioxidant and free radical scavenging effects of the plant and presence of flavonoids, tannins and other phenolic compounds in the extract. Therefore, the observed antidiabetic activity of the ethanol extract of *Anchomanes difformis* may be attributed to the phytoconstituents such as tannins, phlobatannins and flavonoids. The plant extract may be exerting its antidiabetic activity through free radical scavenging and antioxidant activities as it has been reported by[8] that the root extracts of *Anchomanes difformis* possessed a strong free radical scavenging and antioxidant activity. The results of this study have shown that the ethanol extract of *Anchomanes difformis* leaves possess antidiabetic activity. Further studies could be done to isolate and characterise the active principle for pharmacological evaluation.

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