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Ethanol Extracts of Roots of *Anchomanes difformis* ENGL Roots as an Antihyperglycemic Agent in Diabetic Rats

O. Adeyemi[1, *], T. T. Makinwa[2], R. N. Uadia[2]

[1]Department of Environmental science, Federal University of Petroleum Resources; Effurun, Delta State, Nigeria
[2]Department of Biochemistry, University of Benin, Benin City Nigeria

Abstract

Effects of ethanolic root extracts of *Anchomane difformis ENGL* of the family Araceae on normal and alloxan–induced diabetic rats were investigated in 4 groups of rats (6 rats per group). A dose of extract (500mg/kg) was orally administered twice daily to experimental rats for 3 consecutive days. The levels of plasma glucose, urea, creatinine and cholesterol were significantly ($p<0.05$) increased in diabetic rats following the induction of diabetes. In contrast, there was a significant ($p<0.05$) decrease in plasma protein and plasma albumin concentrations of diabetic rats compared with normal control rats. Administration of ethanolic extract of *Anchomanes difformis ENGL* significantly lowered the plasma glucose level ($p<0.05$) of the diabetic treated rats compared with the diabetic control groups. This decrease is also accompanied by a significant decrease in the plasma urea, creatinine and cholesterol concentrations ($p<0.05$). The plasma protein and plasma albumin level were brought back to normal. However administration of the plant extract did not show any hypoglycemic effect on normal rats ($p>0.05$) compared with the control. The results suggest that extract of *Anchomanes difformis ENGL* is antihyperglycemic and not hypoglycemic with a great potential to attenuate diabetes in rat by a mechanism that suppresses hepatic gluconeogenesis.

Keywords

Anchomanes difformis ENGL, Antihyperglycemic, Diabetic Rats, Ethanol, Extracts, Root

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1. Introduction

Diabetes mellitus is a constellation of abnormalities caused by insulin deficiency and/or insulin action, which result in impaired metabolism of glucose, lipid and protein[1]. Diabetes is characterized by polyuria, polydipsia, weight loss in spite of polyphagia (increased appetite), hyperglycemia, ketosis, acidosis and coma. If the disease is not adequately controlled, serious long term complications may arise which include; cardiovascular disease, retinal damage, nerve damage, chronic renal failure and microvascular damage, which may cause impotency and poor wound healing[2].

Although all forms of diabetes have been treatable since insulin became medically available in 1921, but there is still no cure[3]. Diabetes is likely to remain a significant threat to public health in years to come, in the absence of effective and affordable interventions, the frequency of the disease will escalate world wide with a major impact on the population of developing countries[4]. Therefore since diabetes is progressing unabated there is an urgent need to identify indigenous natural sources of active substances against the disease. Undoubtedly medicinal plants are relevant in both developing and developed nations of the world as sources of drugs and herbal extracts of various chemotherapeutic purpose[5]. Recently, some plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipiddemic remedies[6]. Most of

* Corresponding author
E-mail address: adeyemi.olalekan@fupre.edu.ng (O. Adeyemi), makinwatope@yahoo.com (T. T. Makinwa)

these plants have been reported to contain glycosides alkaloid terpenoids, flavonoids, caroteniods etc that are frequently implicated as having antidiabetic effect[7].

Anchomanes difformis ENGL of the family araceae is commonly known as Abirisako in the south west of Nigeria[8]. The rhizome has been used for the treatment of many disease conditions in various parts of the world, it is used as rubefacients vesicant for external application in Guinea, in Ivory coast it is considered to be a powerful purgative and is used to treat Oedema, difficult child birth, as poison antidote as well as a strong diuretic for treating urethal discharge, jaundice and kidney pains[9]. Phytochemical analysis of the leaf, stem and tuber of *Anchomanes difformis* revealed the presence of saponins, tannins and alkaloids[10]. Recent findings also revealed that ethanol extracts of the stem, leaves and tuber of *Anchomanes difformis ENGL* contains saponins tannins and alkaloids. It was revealed that the tuber extract is an effective inhibitor of salmonella species and bacillus substillis. This confirms the local use of the extract in the treatment of dysentery by herbal practitioner[8]. Other studies revealed that methanolic extract of *Anchomanes difformis* is trypanocidal[11]. Hence in this study the ethanol root extract of *Anchomane difformis ENGL* was evaluated for the potential antidiabetic effect on alloxan induced diabetes rats. This study evaluated the effect of daily oral administration of the extract on glycemic control in normal and diabetic rats as well as on indices of diabetes disease such as; lipid profile and renal dysfunction markers (urea and Creatinine) as well as protein and albumin.

2. Materials and Methods

Alloxan was purchased from sigma chemical company USA. All kits used for biochemical analysis were obtained from Randox laboratory Ltd Ardmore, Diamond road Crumlin, Co. Antrim, United Kingdom.

2.1. Plant material and Extraction

Fresh plant of *anchomanes difformis* was collected from a cocoa plantation, at Alade ldanre town, in Ondo state Nigeria, in the month October 2013. The plant was identified and authenticated by Dr. Aigbokan at the Herbarium of the Botany Department, University of Benin, Benin City, Nigeria.

The plant tuber was separated from the plant. The tuber was further sliced into pieces to aid sun drying, the tuber was sun dried and ground into powder. The powder (100g) was soaked in 500 mls of ethanol for sixty hours. The extract was sieved and the filtrate was concentrated by evaporation in a water bath at (80^0C) into a semi solid form. The extract weighing 20g was reconstituted by dissolving the residue in 100 ml of distilled water, which was refrigerated at 4^oc until required.

2.2. Experimental Rats

Mixed sex albino wistar rats weighing 150-250g, bred in the animal house, Department of pharmacology, University of Benin, Benin City were used. The animals were acclimatized for a period of two weeks and fed a pellet diet and water *ad libitum*. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Experimental animals, fasted overnight, received a freshly prepared solution of 100mg of alloxan per kg body weight intraperitoneally. Rats are fed 5% stock solution of glucose for 24h. After 2 days of alloxan administration, rats showing mild diabetes with hyperglycemia (i.e blood glucose levels of 140 -150mg/dl) were used for the experiment. Blood samples were tested using one touch glucometer.

Twenty four mixed sex wistar rats were used in this study. The rats were randomized and divided into four groups of six animals each.

Group 1 – normal control rats

Group 2 – normal rats receiving *Anchomanes difformis* (500mg/kg body weight) in solution twice daily by gavage

Group 3 – diabetic control rats

Group 4 – diabetic rats receiving *Anchomanes difformis* (500mg/kg body weight) in solution twice daily by gavage.

The experimental animals were acclimatized for a period of fourteen (14) days, after which blood samples were drawn by tail vein puncture on day 15 for the determination of baseline parameters, followed by the induction of diabetes into some of the animals. Blood samples were drawn on day 20 after the induction of diabetes (pretreatment) and afterwards on day 22, 24 and 26 post–treatment. Bloods were collected into vials containing sodium flouride and EDTA for the determination of blood glucose and of other blood parameters respectively. The samples collected were immediately placed on ice and then centrifuged using Gallenkamp Centrifuge at 3500 r.p.m for 10 min to obtain the plasma.

2.3. Biochemical Analysis

The concentration of Blood glucose, plasma urea, plasma cholesterol, plasma albumin, plasma protein and plasma creatinine was estimated by using reagent kit from Randox laboratory Ltd U.K. Blood glucose was estimated using glucose oxidase reaction.

The plasma urea was estimated by the method of Weatherbum[12]. Creatinine in the plasma was estimated using

the method of Barlets and Bohmer[13]. Plasma cholesterol was estimated by method of Trinder[14] using enzymatic hydrolysis and oxidation reaction. Plasma protein was estimated using the method of Tietz[15] using Biuret method. The albumin concentration in the plasma was determined using the method described in the Randox albumin kit. 0.01ml of sample distilled water and standard was dispensed into 3 test-tubes respectively and 3ml of working reagent (Bromocresol green concentrate) was added. The mixture was allowed to stand for 5 minutes at 25°C. The absorbance of the sample was measured against reagent blank at 578nm.

2.4. Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) by employing the method of Steel and Torrie[16]. Significant difference between the treatment means was determined at 95% confidence level using Duncan's multiple range test[17].

3. Results and Discussion

3.1. Results

The results of blood glucose concentration in experimental rats are shown in Table 1. There was a significant ($p<0.05$) increase in blood glucose levels in alloxan-induced diabetic rats (Group 3 and 4) when compared with normal rats. Administration of aqueous extract of *A. difformis* results in a significant ($P<0.05$) reduction in the blood glucose level of the treated rat compared with that of diabetic control rats. However, there was no significant decrease in the plasma glucose level of the normal treated rats.

Table 1. Effect of *A. difformis* ENGL on concentration of plasma glucose of experimental rats (mg/dl)

Group	Basal	Diabetic pre-treatment	Diabetic post treatment		
	Day 15	Day 20	Day 22	Day 24	Day 26
1	56.80±0.01^{a}	57.68 ± 5.28^{a}	56.95±3.28^{a}	58.60±5.01^{ab}	57.98±6.79^{ab}
2	62.83±7.93^{a}	60.66 ± 7.68^{a}	56.61±8.06^{a}	55.92 ± 1.50^{a}	55.69 ± 4.58^{a}
3	58.15±10,57^{a}	129.85±11.18^{b}	138.42±3.13^{b}	145.48±5.84^{c}	157.17±9.40^{c}
4	56.47±10.32^{a}	132.60±10.40^{b}	111.08±2.47^{c}	94.70± .56d	87.21± 2.99^{d}

Results are means of six determinations ± SEM. values in the same column carrying different superscripts are significantly different ($P<0.05$).

A significant difference ($p<0.05$) was observed in the plasma urea level of rats in groups (3 and 4) compared with those in groups 1 and 2 after the induction of diabetes. However, administration of *A. difformis* reduced the concentration of plasma urea of rats in group 3 which was significantly different from those in group 4 ($p<0.05$) as shown in Table 2.

Table 2. Effect of *A. difformis* ENGL on concentration of plasma urea (mg/dl)

Group	Basal	Diabetic pre-treatment	Diabetic post treatment		
	Day 15	Day 20	Day 22	Day 24	Day 26
1	27.29±1.70^{a}	28.76 ± 5.99^{a}	29.64± 3.26^{a}	29.08±3.69^{a}	28.64±4.08^{a}
2	27.30±3.21^{a}	25.66 ± 4.73^{a}	30.42± 5.22^{a}	28.05±3.18^{a}	27.34±3.60^{a}
3	26.75±1.50^{a}	60.83± 11.75^{b}	66.71± 2.37^{b}	67.75±4.38^{b}	67.22±3.00^{b}
4	27.15±3.65^{a}	58.42 ± 9.89^{c}	40.05± 8.94^{c}	35.82±5.10^{c}	30.29±2.84^{a}

Results are means of six determinations ± SEM. Values in the same column carrying different superscripts are significantly different ($p < 0.05$)

Table 3 shows that no significant difference was observed at baseline ($p>0.05$). However there was a significant increase in the creatinine concentration observed in diabetic control groups as compared with the diabetic rats receiving the plant treatment. ($p<0.05$) at the end of the experiment.

Table 3. Effect of *A. difformis* ENGL on concentration of Plasma creatinine (mg/dl)

Group	Basal	Diabetic pre-treatment	Diabetic post treatment		
	Day 15	Day 20	Day 22	Day 24	Day 26
1	0.96 ± 0.06^{a}	1.01 ± 0.21^{a}	1.04 ± 0.11^{a}	1.02±0.13^{a}	1.00±0.14^{a}
2	0.94± 0.11	0.90 ± 0.17^{a}	1.06 ± 0.18^{a}	0.98±0.11^{a}	0.96±0.13^{a}
3	0.97 ± 0.05^{a}	2.13 ± 0.41^{b}	2.33 ± 0.08^{b}	2.37±0.15^{b}	2.35±0.38^{b}
4	0.95 ± 0.13^{a}	1.69 ± 0.35	0.91 ± 0.31^{a}	0.90±0.18^{a}	0.88±0.10^{a}

Results are means of six determinations ± SEM. Values in the same column carrying different superscripts are significantly different ($P < 0.05$).

Table 4 shows the concentration of plasma cholesterol while no significant difference was seen in the plasma cholesterol

levels of the normal control rats and normal treated rats. There was a significant difference in plasma cholesterol between diabetic control and diabetic group treated with *anchomanes difformis* ($p < 0.05$).

Table 4. Effect of *A. difformis* ENGL on concentration of plasma cholesterol (mg/dl)

Group	Basal	Diabetic pre-treatment	Diabetic post treatment		
	Day 15	Day 20	Day 22	Day 24	Day 26
1	64.47±16.78[a]	66.84±16.53[a]	68.28±3.79[a]	64.50±0 .51[a]	65.55± 15.05[a]
2	64.45± 9.03[a]	64.20± 4.33[a]	62.95±8.54[a]	64.95±2.30[a]	65.00 ± 2.30[a]
3	64.84± 7.53[a]	79.28± 6.08[b]	86.42±4.51[b]	97.13±4.36[b]	105.98± 5.00[b]
4	63.69± 5.29[a]	74.10 ± 5.70[b]	70.69±10.18[a]	69.00±4.58[a]	63.26±3.90[a]

Results are means of six determinants ± SEM, values in the same column carrying different superscripts are significantly different ($p < 0.05$).

As shown in Table 5, there was no significant difference in the plasma protein concentrations of all experimental rats at basal level. However, Table 6 presents a significant ($P<0.05$) decrease was observed in both the plasma protein and plasma albumin concentrations of diabetic rats compared with control following the induction of diabetes. Administration of plant extract significantly ($P<0.05$) brought the plasma protein and plasma albumin concentrations to normal.

Table 5. Effect of *A. difformis* ENGL on concentration of plasma protein (g/dl)

Group	Basal	Diabetic pre-treatment	Diabetic post treatment		
	Day 15	Day 20	Day 22	Day 24	Day 26
1	5.76 ± 0.14[a]	4.25 ± 0.84[a]	6.99 ± 0.18[a]	5.45 ± 0.10[a]	5.62 ± 0.70[a]
2	6.03 ± 0.77[a]	4.91 ± 0.79[a]	6.41 ± 0.77[a]	5.38 ± 0.08[a]	5.77 ± 0.48[a]
3	5.35 ± 0.39[a]	4.35 ± 0.54[a]	3.68 ± 0.12[b]	3.11 ± 0.15[a]	3.07 ± 0.18[b]
4	6.07 ± 0.63[a]	4.00 ± 0.64[a]	4.07 ± 0.75[b]	4.57 ± 0.39[c]	4.92 ± 0.63[a]

Results are means of six determinants ± SEM, values in the same column carrying different superscripts are significantly different ($p < 0.05$).

Table 6. Effect of *A. difformis* ENGL on concentration of plasma albumin (g/dl)

Group	Basal	Diabetic pre-treatment	Diabetic post treatment		
	Day 15	Day 20	Day 22	Day 24	Day 26
1	4.50 ± 0.04[a]	4.28 ± 0.25[a]	4.50 ± 0.10[a]	4.93 ± 0.47[a]	4.74 ± 0.58[a]
2	4.15 ± 0.23[a]	4.00 ± 0.15[a]	4.29 ± 0.12[a]	4.28 ± 0.26[a]	4.23 ± 0.09[a]
3	4.29 ± 0.20[a]	4.07 ± 0.10[ab]	3.77 ± 0.27[b]	3.55 ± 0.32[b]	2.77 ± 0.45[b]
4	4.38 ± 0.22[a]	3.64 ± 0.32[bc]	3.68 ± 2.83[b]	4.01 ± 0.14[a]	4.15 ± 0.18[a]

Results are means of six determinants ± SEM, values in the same column carrying different superscripts are significantly different ($p < 0.05$).

3.2. Discussion

The increasing use of medicinal plants as source of anti-diabetic agent is due to the extraction and development of several successful drugs and chemotherapeutic agents from plants as well as their use as traditional rural herbal remedies [18-21]. The present study investigated the hypoglycemic effects of ethanol extract of *A. difformis* on alloxan-induced diabetic rats.

In this study the ethanol extract of *A. difformis* effectively decreased the blood glucose in alloxan-induced diabetic rats compared with the diabetic control rats. This significant reduction in glucose levels observed in groups receiving the plant extracts maybe due to the activity of their principles. The plant has been reported to contain strong alkaloids, saponins and tannins[10,22].

In this study, the significant increase in the plasma urea and creatinine of the diabetic rats maybe due to increased catabolism of proteins and nucleic acids, which results in the formation of non-protein nitrogenous compounds; urea and creatinine. It has been reported that amino acid breakdown in the liver and muscle tissues of diabetic animals results in an increase production of urea and creatinine[23]. Notably, the plasma creatinine and plasma urea levels were brought to normal limit by treatment with *A. difformis* in diabetic rats. Since creatinine and urea are markers of renal dysfunction[24], the renal function was gradually restored after proper control of blood glucose levels in the diabetic rats.

Increase in plasma cholesterol level usually occurs in diabetes, which leads to significant change in lipid metabolism and structure[25]. Experimentally, alloxan-induced diabetic hyperglycemia is accompanied by increase in plasma cholesterol[26]. In this study plasma cholesterol value in diabetic rats treated with *A. difformis* extract was significantly ($p<0.05$) lower than that of diabetic control rats.

In this study, the observed decrease in the plasma protein and albumin level in diabetic rats may be due to increased catabolism of proteins and nucleic acids which leads to increase in the formation of non protein nitrogenous

compounds[23]. It may also be due to increased supply of amino acids for gluconeogenesis.

Although the precise mechanism by which *A. difformis* lowers blood glucose is not clear, this study attempts to elucidate the mechanism. It may be, through increased utilization of peripheral glucose as earlier suggested[27-28]. However the plants did not exhibit hypoglycemic activity in normal animals, which is similar in effect to the action of biguanides (metformin). It has been reported that biguanides do not increase insulin secretion but they rather promote tissue glucose uptake and reduce hepatic glucose output thereby producing antihyperglycemic effect[29] and not hypoglycemic effect.

4. Conclusion

On the basis of the aforementioned results, we conclude that *A. difformis* have a significant antihyperglycemic effect in diabetic rats through suppression of hepatic gluconeogenesis, perhaps, as a consequence of mitochondrial inhibition. Therefore this medicinal plant should be considered an excellent candidate for future studies on diabetes mellitus. On the other hand further studies on the effect of *A. difformis* on mitochondrial respiration rate as was proposed is encouraged.

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