Trop J Nat Prod Res, January 2024; 8(1):6016-6027 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effects of Hydroethanolic Rhizome Extract of *Anchomanes difformis* (Blume) in Type 2 (Streptozocin-Nicotinamide-Induced) Diabetic Rats

Idris O. Isioye[1,2,3], Abidemi J. Akindele[1,2]*, Flora R. Aigbe[1,2], Francis J. Olatoye[1,2], Olubusola Olaleye[2,4], Margaret O. Sofidiya[2,4], Abimbola Sowemimo[2,4], Idowu S. Akande[2,5]

[1]*Department of Pharmacology, Therapeutics & Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, P.M.B. 12003, Lagos, Nigeria.*
[2]*Phytopharmakon Research Group, University of Lagos, Lagos, Nigeria.*
[3]*Department of Health Promotion and Education, Ogun State College of Health Technology, Ilese Ijebu, Ogun State, Nigeria.*
[4]*Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria.*
[5]*Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, P.M.B. 12003, Lagos, Nigeria.*

ARTICLE INFO

Article history:
Received 07 June 2023
Revised 13 December 2023
Accepted 02 January 2024
Published online 01 February 2024

ABSTRACT

Diabetes mellitus remains a huge public health concern. *Anchomanes difformis* Blume is an herbaceous plant with numerous folkloric uses. This study investigated the acute and sub-acute effects of hydroethanolic rhizome extracts of *A. difformis* (AD) on glucose level in normal and diabetic Sprague-Dawley rats. Type 2 diabetes was induced with streptozotocin (65 mg/kg) administration after nicotinamide (120 mg/kg) i.p. Diabetic rats were divided into five groups: diabetic control (distilled water 10 mL/kg, p.o.), *A. difformis* (125, 250 and 500 mg/kg, p.o.) and glibenclamide (2.5 mg/kg, p.o.). Group 6 was normal control (non-diabetic; distilled water 10 mL/kg, p.o.). Treatments were done for 21 days. Body weight and fasting blood glucose level of the animals were determined weekly. On day 21, blood samples were collected for serum biochemical and lipid profile assays. Vital organs were collected for assay of tissue antioxidant levels and histopathological assessment. AD produced significant ($P<0.05$-0.001) dose-dependent reduction in blood glucose level with peak effects at 500 mg/kg dose on day 21. 500 mg/kg AD elicited significant increase in the levels of SOD and CAT in the liver, kidney and pancreas. The level of ALP was significantly reduced in diabetic rats by AD (250-500 mg/kg). 250-500 mg/kg AD significantly reduced the levels of creatinine and urea, with significant increase in total protein levels. 500 mg/kg AD significantly reduced ($P<0.05$) cholesterol level. No significant histopathological distortions were observed in vital organs. The effects of AD extract were comparable to that of glibenclamide. Findings in this study suggest that AD possesses significant antidiabetic effect.

Keywords: *Anchomanes difformis*, Antihyperglycaemic, Diabetes mellitus, Antidiabetic, Antioxidant activity, Hepatoprotective

Introduction

Without doubt, diabetes mellitus (DM) is and has become a huge public health issue in both economically developed and economically developing nations. DM is one of the four priority non-communicable diseases targeted by world health leaders and recommended to be actively prevented and focused on by the World Health Organization (WHO). Despite this public health focus, the global prevalence of DM continues to soar.

In 2021, it was estimated that about 537 million adults (20-79 years) are living with diabetes, with the projection that the number of people living with diabetes will rise to 643 million by 2030 and 783 million by 2045.[1]

*Corresponding author. E mail: jakindele@unilag.edu.ng; phytopharmakonunilag@gmail.com
Tel: +2348062359726

Citation: Isioye IO, Akindele AJ, Aigbe FR, Olatoye FJ, Olaleye O, Sofidiya MO, Sowemimo A, Akande IS. Effects of Hydroethanolic Rhizome Extract of *Anchomanes difformis* (Blume) in Type 2 (Streptozocin-Nicotinamide-Induced) Diabetic Rats. Trop J Nat Prod Res. 2024; 8(1):6016-6027. http://www.doi.org/10.26538/tjnpr/v8i1.44

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Furthermore, the report estimated that diabetes caused 6.7 million deaths and about USD 966 billion in health expenditure. The global prevalence of DM is expected to rise 10.2% by 2030 and 10.9% by 2045 with a disproportionately higher prevalence in urban areas.[2]

It is therefore not shocking that the amount of resources and research channelled into reducing this global public health concern remains huge as its prevalence continues to rise. The reason for this is not far-fetched either. Firstly, DM presents with complications; it is known that DM increases the risk of heart attacks and strokes by a factor of 2 to 3 in adults,[3] is a dominant cause of global blindness causing 2.6% of global blindness[4] and is the principal cause of kidney failure.[5] Secondly, the socio-economic impact of DM may be unrivalled compared to other non-communicable diseases. It is estimated that people with DM have more than double the mean unadjusted costs (USD 12,180 vs. 5,058) when compared with people who do not have DM,[6] meaning people with DM have significantly higher direct incremental costs than those without DM. Furthermore, the medical treatment costs for DM is estimated to be 2 to 8-fold higher than other chronic conditions[7] with inpatient hospitalization due to complications accounting for a sizeable portion of these costs.[8] Additionally, doctors office visit, inpatient hospital stay and prescription medications were 1.9, 2.6 and 3.4 times respectively higher in patients with DM compared to people without DM.[9] All of these suggest that DM presents a huge economic burden in both economically developed and developing nations.

However, compared to economically developed countries, the prevalence of DM is growing much rapidly in developing countries

which includes both the low- and middle-income countries.[10] In the African region, the high blood glucose age standardized mortality rate per 100,000 people is 111.3 - a figure which is third only to the Eastern Mediterranean (139.6) and South-East Asia (115.3) regions respectively.[10] Specifically, the standardized mortality rate per 100,000 people in the African region is approximately twice those of the European (55.7) and Western-Pacific/American (67) regions. It can thus, be inferred that the medical and economic burden of DM in the African region is significantly higher than the western nations.

While both Type 1 and Type 2 DM are generally prevalent, most of the world's diabetic cases are Type 2 DM,[11] with Type 1 DM more common in children.[10] DM is undoubtedly a chronic disease which requires long term medical treatment; however, it is largely preventable and can be properly managed to mitigate some of its complications earlier stated and improve quality of life of patients. Type 2 DM has both non-modifiable risk factors (which include genetics, ethnicity and age) and modifiable risk factors (which include obesity, overweight, insufficient physical activity, smoking and unhealthy diet). These risk factors have important implication in the management of the disease.

Like cardiovascular diseases, DM is yet to be reported as curable[12] and can only be managed by several medical and lifestyle interventions. While a combination of both medical and lifestyle intervention is often recommended by the healthcare providers, diet and physical activity have been suggested to be more effective than medication.[10] Regardless of this, the mainstay of the management of DM seems tilted more towards medication and several orthodox medications have been developed to combat and manage DM. Many of these orthodox medications have been largely successful and include but are not limited to different class of anti-diabetic and hypoglycaemic drugs, including sulphonylureas, meglitinides, alpha-glycosidase inhibitors etc.

Drugs derived from plant sources have become increasingly important and popular in the management of DM, as well as in the research into newer effective drugs with lesser side effects for the management of DM. More than 800 plants have been reported to have antidiabetic activity in the Indian literature[13] and more than 1200 plants have been identified to possess hypoglycaemic activity from ethno-pharmacological surveys.[14]

Native to the African continent, *Anchomanes difformis* Blume (Araceae) is an herbaceous plant with numerous folkloric and pharmacologically proven medicinal uses.[15] Several parts of the *A. difformis* (AD) plant have been reported for their medicinal uses and these include the rhizome, tuber/roots, leaves and stems.[16] Traditionally, a concoction of the leaves of AD has been used as an antibacterial agent, specifically against *Staphylococcus aureus*,[17] in the amelioration of pain and inflammation[18] and in the treatment of asthma, ulcer and cough.[16] In some countries and cultures such as Ivory Coast, the rhizomes of AD is used as a poison antidote, a purgative, a diuretic and in the treatment of oedema, jaundice and kidney pains.[19] In Nigeria, a decoction made from the peeled roots of AD has been used in the treatment of dysentery and diarrhoea.[20] Scientifically and pharmacologically, the analgesic and anti-inflammatory,[18] anti-ulcer,[21,22] anti-asthmatic[23], anti-diabetic,[13,24] anti-microbial,[25] anti-onchocercal,[26] antioxidant[15] and insecticidal[27] effects of AD have been proven and documented in literature.

This present study was designed to investigate the acute and sub-acute effects of the hydroethanolic rhizome extracts of *Anchomanes difformis* on glucose level in normal, glucose loaded and diabetic rats, as well as the biochemical, histopathological and antioxidant effects of AD in Type 2 diabetic rats.

Materials and Methods

Drugs and chemicals

Methanol, ethanol (NAAFCO Scientific Supplies Industry, Lagos, Nigeria), streptozotocin, nicotinamide (Sigma Chemical Co., St. Louis, USA), glibenclamide (May & Baker, Lagos, Nigeria), glucose (Abbachem Tradings Limited, Lagos, Nigeria), glucometer and glucometer strip (Roche Diagnostics, Basel, Switzerland) were procured from a local supplier.

Plant materials and extraction

Fresh rhizomes of AD were harvested from a farm in Ikire, Oyo State, Nigeria in October 2016 and authenticated in the Department of Botany, University of Lagos, Nigeria (LUH 6110). The rhizomes were then sliced, chopped into tiny bits and air-dried until constant weight was obtained after which they were ground to powder. The powdered material (500 g) was macerated in hydroethanol (ethanol and water; 70:30; 4 L) for 72 h with intermittent stirring. The extract was decanted and filtered. The residue was re-macerated twice for exhaustive extraction and the combined filtrate was evaporated to dryness at 40ºC in a laboratory oven. The yield (10.7%) was calculated as a percentage of the derived weight of the dried extract to the weight of the starting material. The extract obtained was observed to be brownish in colour with a powdery crystalline texture. It was soluble in water with a pH of about 6.3. A solution of the extract was prepared by dissolving 25 g of AD rhizome extract in 25 mL of methanol giving a 1 mg/mL concentration used for phytochemical estimations carried out in this study. Several other working concentrations of AD were prepared for experimentations with distilled water as vehicle.

Phytochemical estimations

The phytochemical analysis of AD extract includes the determination of total flavonoids, total proanthocyanidins and total phenolic content in the extract. Using the extract solution prepared for phytochemical estimations, total flavonoids was determined as described by Ordonez *et al.*,[28] total proanthocyanidins was assessed using the procedure of Aziz *et al.*[29] and lastly, total phenolic content was estimated using the modified Folin-Ciocalteu method of Wolfe *et al.*[30]

Experimental animals

Ninety-six (96) healthy male Sprague-Dawley rats with weights between 100-200 g and 10 mice with weights between 15-25 g were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. The animals were maintained under standard laboratory conditions (23–25ºC, 12 h/12 h light/dark cycle), fed with standard rodent diet (Livestock Feeds PLC, Lagos, Nigeria), provided with water as needed and allowed to acclimatize to the new environment for 2 weeks before start of experimentation. The procedures adopted in this study were in compliance with the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals, 8th edition (Committee for the Update of the Guide for the Care and Use of Laboratory Animals). Ethical approval was obtained from the Health Research Ethics Committee (HREC) of the College of Medicine, University of Lagos (CMUL), Nigeria, with reference number CM/HREC/010/16/063.

Experimental Design

Acute toxicity test

Ten (10) mice were separated into two groups of 5 mice each and were fasted overnight before the acute toxicity test was carried out. For the acute toxicity test, one group received distilled water while the second group received 5000 mg/kg dose of AD extract. Both groups were then observed for 2 hours after administration to assess behavioural changes such as writhing, grooming, rearing, increase/decrease locomotion, pilo-erection, sedation/aggressiveness, response to touch, defecation, convulsion etc. Mortality rate after 24 h was determined, and the surviving mice were observed for further 14 days for any signs of delayed toxicity.

Determination of blood glucose levels

Fasting blood glucose (FBG) level was determined using the glucometer strips and glucometer. Blood was collected at predefined time intervals by nipping the animal's tail with a lancet and squeezing 2-3 drops of fresh blood on the glucometer strips which was then inserted into a glucometer and the displayed blood glucose values recorded.

Normal glucose and oral glucose tolerance test in normal and glucose – loaded rats (NG-OGTT)

Thirty (30) animals randomly distributed into 5 groups of 6 rats each were fasted overnight and their FBG level was determined and

recorded. The weight of the animals in all 5 groups was determined and recorded. Animals in Group 1 received distilled water (10 mL/kg) while animals in Group 2 received glibenclamide at a dose of 2.5 mg/kg per day. Animals in Groups 3, 4, and 5 received AD extract at doses of 125, 250 and 500 mg/kg respectively. After initial administration, blood glucose levels were determined at 30, 60, 90 and 120 min. The animals in all groups were then administered orally with 2 g/kg of glucose and blood glucose level was again determined after treatment at 150, 180, 240, 300, 360 and 420 min according to the modified method of Orhan *et al.*[31]

Induction of diabetes mellitus
Diabetes mellitus was induced in the animals by single intraperitoneal (i.p.) administration of 65 mg/kg streptozotocin (STZ) 15 min after the i.p. administration of 120 mg/kg nicotinamide dissolved in 0.9% saline solution.[32] STZ was diluted in recently prepared sodium citrate buffer prior to injection to avoid degradation. Fasting blood glucose level of test animals was determined after 72 h. Rats with blood glucose level ≥ 200 mg/dL were considered to be diabetic.

Acute antidiabetic effects of test sample
Acute antidiabetic effect of AD and glibenclamide was conducted according to the method of Aslan *et al.*[33] Thirty (30) diabetic rats were weighed and randomly allocated to 5 groups (n=6). While Group 1 received distilled water (10 mL/kg) only, Group 2 received 2.5 mg/kg glibenclamide and Groups 3, 4, and 5 received AD extract at doses of 125, 250 and 500 mg/kg respectively. Blood glucose level was determined for all animals in all 5 groups at 30, 60, 120, 240 and 360 min post-treatment.

Sub-acute antidiabetic effects of test sample
Thirty (30) diabetic rats were randomly allocated to 5 groups (n=6). Six (6) non-diabetic rats were assigned to Group 6 (negative control). Group 1 and 6 received distilled water (10 mL/kg) only, Group 2 received 2.5 mg/kg glibenclamide and Groups 3, 4, and 5 received AD extract at doses of 125, 250 and 500 mg/kg respectively daily for 21 days. Fasting blood glucose level and weight of animals were determined on days 1, 7, 14 and 21. On day 20, all animals were fasted overnight and on day 21, blood samples were collected through the retro-orbital sinus for biochemical parameters assessment. Furthermore, the rats were sacrificed through cervical dislocation under anaesthesia. Kidney, liver and pancreatic tissues were harvested, washed, weighed and processed for determination of antioxidant indices and histopathological assessment.

Sub-acute effect in normoglycaemic rats
Thirty (30) normoglycaemic rats were randomly assigned to 5 groups (n=6). Group 1 received distilled water (10 mL/kg), Group 2 received 2.5 mg/kg glibenclamide and Groups 3, 4, and 5 received AD extract at doses of 125, 250 and 500 mg/kg respectively daily for 21 days. Fasting blood glucose level and weight of animals were determined on days 1, 7, 14 and 21.

Biochemical and lipid profile assessment
Blood samples from the diabetic test animal groups were collected into plain bottles, allowed to coagulate for 30 min and serum was separated through centrifugation. Serum samples were analysed for aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), albumin (ALB), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), creatinine, total bilirubin (TB) and urea using Roche and Cobas commercial kits and Roche/Hitachi 904 automated analyser (Roche Diagnostics, Basel, Switzerland).

Estimation of in vivo antioxidants and MDA levels
The levels of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) in the harvested organs were estimated using established protocols as reported by Akindele *et al.*[34]

Histopathological assessment
The harvested kidney, liver and pancreas were fixed in 10% formal-saline. They were dehydrated in graded alcohol, embedded in paraffin, cut, stained and viewed under the microscope in accordance with the protocol reported by Ishola *et al.*[35]

Statistical analysis
Data are expressed as mean ± standard error of mean (SEM). Statistical analysis was done using one-way ANOVA with Tukey's multiple comparison test using GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Results were considered significant at $P<0.05$.

Results and Discussion

Phytochemical analysis of Anchomanes difformis (AD)
The hydroethanolic extract of AD rhizomes was found to contain significant amounts of flavonoids, proanthocyanidins and phenols (Table 1). This finding is consistent with literature in which the ethanolic and methanolic extracts of leaves of AD were reported to contain several phytochemicals such as glycosides, tannins, flavonoids, saponins and reducing sugars.[13,36] The ethanolic extract of the roots of AD has been reported to contain phenols, saponins, tannins and alkaloids.[24,36] The aqueous extract of AD has been reported to contain other phytochemicals such as rutin, quercetin, phloridzin, chlorogenic acids, and kaempferol which are reported to be active against hyperglycaemia, oxidative stress, inflammation, and apoptosis.[37-39] This may partly explain the antihyperglycaemic and antidiabetic effects of AD. Additionally, the importance of oxidative stress in the pathophysiology of DM is well documented[33] and literature is replete with antioxidant effects of flavonoids and proanthocyanidin, an oligomeric flavonoid[40-43] found in AD extract.

Table 1: Concentration of phytochemicals in *Anchomanes difformis* rhizome extract

Phytochemicals	Concentration (mg/dL)
Total flavonoid content	13.364 mg/g
Total proanthocyanidin content	14.760 mg/g
Total phenolic content	9.170 mg/g

Table 2: Effect of *Anchomanes difformis* extract on normal blood glucose level (mg/dL)

Treatments	0 min	30 min	60 min	90 min	120 min
Normal control	83.13 ± 2.52	79.50 ± 2.37	77.00 ± 3.64	71.63 ± 5.16	65.37 ± 6.83
125 mg/kg AD	66.00 ± 5.46	70.00 ± 2.39	73.63 ± 3.32	67.75 ± 2.84	63.13 ± 2.63
250 mg/kg AD	66.00 ± 5.46	70.00 ± 3.31	73.63 ± 3.31	67.75 ± 2.83	63.13 ± 2.63
500 mg/kg AD	73.62 ± 3.88	67.50 ± 5.62	62.62 ± 5.37	53.12 ± 2.38	50.13 ± 2.64
Glibenclamide	85.63 ± 3.41	69.40 ± 5.07	52.65 ± 5.00*	41.13 ± 2.39**	35.13 ± 2.21***

Values are mean±SEM (n=6). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. normal control.

One-way ANOVA followed by Tukey's multiple comparison test. AD: *Anchomanes difformis.*

Table 3: Effect of *Anchomanes difformis* extract on blood glucose level (mg/dL) in glucose loaded rats

Treatments	120 min	150 min	180 min	240 min	300 min	360 min	420 min
Normal control	65.37 ± 6.83	86.25 ± 2.49	141.80 ± 5.11	103.12 ± 7.87	79.13 ± 3.80	71.75 ± 2.68	72.12 ± 2.67
125 mg/kg AD	63.13 ± 2.63	76.25 ± 2.5	135.87 ± 5.11	96.50 ± 7.87	77.37 ± 3.80	65.62 ± 2.68	51.00 ± 3.04
250 mg/kg AD	63.13 ± 2.63	73.75 ± 2.91	133.37 ± 1.17	97.25 ± 6.96	63.75 ± 4.07	53.50 ± 2.67	44.87 ± 2.27
500 mg/kg AD	40.13 ± 2.64	73.87 ± 4.25	137.75 ± 7.46	84.00 ± 5.47	76.75 ± 4.52	66.62 ± 2.81	59.25 ± 1.62
Glibenclamide	40.13 ± 2.64	48.37 ± 2.92	102.12 ± 11.65	56.00 ± 4.20	37.75 ± 3.88	33.12 ± 4.24	30.75 ± 4.77

Values are mean±SEM (n=6). $P>0.05$. One-way ANOVA followed by Tukey's multiple comparison test. AD: *Anchomanes difformis.*

Table 4: Effect of *Anchomanes difformis* extract on blood glucose level in normoglycaemic rats

Treatments	Day 1 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)	Day 21 (mg/dL)
Normal control	68.25 ± 4.20	73.38 ± 3.50	81.13 ± 2.20	73.75 ± 3.38
125 mg/kg AD	74.25 ± 3.56	75.63 ± 3.20	74.75 ± 5.20	78.62 ± 3.80
250 mg/kg AD	73.75 ± 3.09	74.00 ± 2.50	71.17 ± 1.17	65.37 ± 2.82
500 mg/kg AD	72.87 ± 5.10	72.88 ± 3.80	65.14 ± 3.21	51.13 ± 2.87**
Glibenclamide	65.50 ± 2.63	43.00 ± 2.63*	40.50 ± 2.86*	32.33 ± 1.41***

Values are mean±SEM (n=6). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. normal control. One-way ANOVA followed by Tukey's multiple comparison test. AD: *Anchomanes difformis.*

Effect of Anchomanes difformis (AD) in acute toxicity test

The hydroethanolic extract of AD did not produce any mortality after administration of up to 5000 mg/kg to test animals. There were no visible signs of delayed toxicity immediately after administration and 14 days post-administration. Also, no mortality was observed immediately after administration, 24 h and 14 days post-administration. This indicates that AD is well tolerated and may not pose any acute risk. This result is consistent with the findings of Aderonke *et al.*[13] and Ovuakporie-Uvo and Idu[44] in which acute doses ≥5000 mg/kg resulted in no adverse event or mortality in the test animals.

Effect of Anchomanes difformis (AD) in normal and diabetic rats

Current literature is replete with studies on the antihyperglycaemic and antidiabetic activity of several plant parts of *A. difformis* mainly in Type 1 models of diabetes. The antihyperglycaemic activity of the ethanolic extract of the tuber or roots[24] and leaves[13] of AD has been reported. However, no hypoglycaemic effect was reported with the aqueous extract of the leaves of AD[24]. This study was designed to appraise the antihyperglycaemic and antidiabetic effects of the hydroethanolic extract of the rhizome of AD in Type 2 diabetic rats and to investigate the acute antihyperglycaemic effect of AD in normal, glucose loaded and diabetic rats.

Effect of Anchomanes difformis (AD) on normal blood glucose level and in glucose loaded rats

A. difformis at all tested doses did not elicit any significant change in blood glucose levels at the various time intervals. However, there was a dose-dependent non-significant reduction in blood glucose levels at the 30, 60, 90 and 120 min time intervals. Glibenclamide, on the other hand, produced a significant reduction in blood glucose levels at the 60 ($P<0.05$), 90 ($P<0.01$) and 120 ($P<0.001$) min time intervals. The peak effect was recorded at the 120 min interval (Table 2).

The administration of 2 g/kg body weight of oral glucose led to an expected elevation of blood glucose level after 30 min. This, however, peaked after another 30 min followed by gradual decrease in blood glucose level. This trend was observed for all the groups in which the blood glucose levels peaked after 60 min of administration of oral glucose to the animals. There was no significant decrease in the blood glucose levels across all AD and glibenclamide doses and across all time intervals (Table 3). In this study, the experimental and acute induction of hyperglycaemia in normoglycaemic animals by administration of 2 g/kg glucose was consistent with literature[33] in which hyperglycaemia was induced in animals 30 min after administration of high dose of glucose. Hyperglycaemia, however, peaked 60 min after administration in all groups, including the glibenclamide group. Generally, there was a non-significant dose-dependent diminution in blood glucose level with AD across all doses and across all the time intervals. 250 and 500 mg/kg AD, however, generally seemed to produce a more consistent reduction in blood glucose levels in comparison with 125 mg/kg AD dose. Glibenclamide produced similar non-significant reduction in blood glucose levels in glucose loaded rats with its effect being more prominent than all the doses of AD. In normoglycaemic rats (with no diabetes or hyperglycaemia induced), there was a non-significant dose-dependent acute (0 - 60 min) reduction in the blood glucose levels across all doses of AD consistent with the effect produced with glucose loaded animals.

Sub-acute effects of Anchomanes difformis (AD) in normoglycaemic rats

A. difformis at a dose of 500 mg/kg elicited a significant diminution ($P<0.01$) in blood glucose level on day 21 but elicited a non-significant decrease in glucose level on days 7 and 14. On days 14 and 21, AD at 125 and 250 mg/kg doses elicited non-significant diminution in blood glucose levels. *A. difformis* at all doses produced a non-significant change in blood glucose levels on days 1 and 7. Glibenclamide, on the other hand, elicited notable diminution in the blood glucose levels on day 7 ($P<0.05$), day 14 ($P<0.05$), and day 21 ($P<0.001$). Overall, glibenclamide produced a more significant decrease in blood glucose level versus AD at all doses (Table 4). *A. difformis* at all doses and glibenclamide at 2.5 mg/kg dose produced a non-significant change in the body weight of all animals across all groups and across all day intervals (days 1, 7, 14 and 21) (Table 5).

A. difformis across all doses, except AD 500 mg/kg, elicited a non-significant decrement in blood glucose levels in normoglycaemic rats over the duration of 21 days. 500 mg/kg AD, however, elicited a significant diminution in blood glucose level in normoglycaemic animals on day 21 only. This finding is in contrast with that of Adeyemi *et al.*[24] in which 21 days administration of 500 mg/kg AD did not elicit any hypoglycaemic effect in normoglycaemic rats and with that of Ovuakporie-Uvo and Idu[45] in which AD at dose as high as 2500 mg/kg for 21 days failed to elicit a significant decrease in blood glucose levels

in normoglycaemic rats. Based on these findings, it is possible to hypothesize that AD possibly works more as an antihyperglycaemic agent in the presence of high blood glucose level than as a hypoglycaemic agent. In the acute and sub-acute antidiabetic test in normoglycaemic animals, glibenclamide elicited a significant reduction in blood glucose level after 1 h of administration and consistently from day 7 to 21, confirming its hypoglycaemic effect.

The administration of AD to test animals at a dose of 125 - 500 mg/kg did not elicit any significant change in the body weight of the normoglycaemic (as well as the diabetic) rats throughout the duration of experimentation. However, Aderonke *et al.*[13] reported a non-significant decrease in the weight of diabetic animals treated with 250 - 500 mg/kg dose of AD. In this study, AD did not elicit such decrease in weight of test animals for both normoglycaemic and diabetic animals. However, it is important to state that Aderonke *et al.*[13] used the ethanolic extract of AD leaves in their study; this may account for the observed disparity in effects. This study used the hydroethanolic extracts of the rhizomes of AD.

Acute effect of Anchomanes difformis (AD) on basal blood glucose level in diabetic rats

A. difformis at 500 mg/kg dose elicited a notable decrease in the levels of blood glucose at 60 min (*P<0.05*), 120 min (*P<0.001*), 240 min (*P<0.001*) and 360 min (*P<0.001*) after administration in comparison with the diabetic control group rats which were not administered any test drug. Similarly, AD at 250 mg/kg elicited significant diminution in blood glucose levels at 120 min (*P<0.05*), 240 min (*P<0.01*) and 360 min (*P<0.01*) after administration. However, AD at 125 mg/kg dose produced a non-significant change in blood glucose level after administration to diabetic rats. Glibenclamide produced significant decrements in the blood glucose level at 60 min (*P<0.01*), 120 min (*P<0.001*), 240 min (*P<0.001*) and 360 min (*P<0.001*) post-administration. Overall, glibenclamide produced a more significant decrease in basal blood glucose level versus all doses of AD (Table 6). The induction of DM with nicotinamide-STZ (model of Type 2 diabetes) led to a consistent increase in blood glucose levels above 200 mg/dL in rats consistent with literature[32,33,46]. Consistent with the hypothesis that AD possibly works more as an antihyperglycaemic, AD at experimented doses of 250 and 500 mg/kg led to significant decrease in basal blood glucose levels in diabetic rats from 60 min after administration of AD, with the most prominent effect at the 500 mg/kg dose.

Sub-acute effect of Anchomanes difformis (AD) in diabetic rats

After 14 days of administration, AD at all doses produced significant diminution in the levels of blood glucose in diabetic rats (*P<0.05*, 125 and 250 mg/kg; *P<0.01*, 500 mg/kg versus diabetic control respectively). Similarly, after 21 days of administration, AD at all doses elicited a notable decrease in blood glucose levels in diabetic rats (*P<0.01*, 125 mg/kg; *P<0.001*, 250 and 500 mg/kg versus diabetic control respectively). On days 1 and 7, AD at all doses produced non-significant change in blood glucose levels. Glibenclamide, on the other hand, elicited significant diminution in glucose levels on day 7 (*P<0.05*), day 14 (*P<0.01*) and day 21 (*P<0.001*) (Table 7). *A. difformis* at all doses and glibenclamide at 2.5 mg/kg dose produced a non-significant change in the body weight of all animals across all groups and across all day intervals (days 1, 7, 14 and 21) (Table 8).

Table 5: Effect of *Anchomanes difformis* extract on body weight of normoglycaemic rats

Treatments	Day 1	Day 7	Day 14	Day 21
Normal control	133.30 ± 2.03	138.50 ± 2.29	149.20 ± 2.30	167.70 ± 1.23
125 mg/kg AD	151.84 ± 4.43	147.27 ± 3.36	155.41 ± 4.15	160.74 ± 4.05
250 mg/kg AD	140.30 ± 2.60	146.20 ± 1.89	154.75 ± 2.65	168.00 ± 1.49
500 mg/kg AD	138.54 ± 2.01	145.35 ± 2.19	154.65 ± 1.80	166.75 ± 1.85
Glibenclamide	151.55 ± 2.44	157.60 ± 2.40	143.55 ± 2.07	152.27 ± 2.51

Values are mean±SEM (n=6). *P*>0.05. One-way ANOVA followed by Tukey's multiple comparison test.
AD: *Anchomanes difformis.*

Table 6: Acute effect of *Anchomanes difformis* extract on basal blood glucose level in diabetic rats

Treatments	Initial BGL (mg/dL)	30 min (mg/dL)	60 min (mg/dL)	120 min (mg/dL)	240 min (mg/dL)	360 min (mg/dL)
Diabetic control	253.00 ± 27.54	248.00 ± 24.70	254.25 ± 31.04	264.5 ± 29.00	272.25 ± 29.08	276.00 ± 26.50
125 mg/kg AD	197.50 ± 18.98	214.50 ± 24.58	216.25 ± 23.92	205.75 ± 20.75	201.25 ± 17.67	200.25 ± 26.30
250 mg/kg AD	245.00 ± 43.14	196.50 ± 27.30	167.25 ± 23.86	157.25 ± 20.40*	145.50 ± 21.88**	136.50 ± 21.46**
500 mg/kg AD	226.50 ± 23.51	196.00 ± 25.28	152.75 ± 16.61*	122.50 ± 14.22***	111.00 ± 16.91***	100.04 ± 18.42***
Glibenclamide	256.75 ± 36.41	200.00 ± 32.63	164.00 ± 25.90**	142.00 ± 22.72***	133.75 ± 26.73***	107.50 ± 21.25***

Values are mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 vs. diabetic control. One-way ANOVA followed by Tukey's multiple comparison test.
AD: *Anchomanes difformis.* BGL: Baseline glucose level.

Table 7: Effect of *Anchomanes difformis* extract on blood glucose level in diabetic rats

Treatments	Day 1 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)	Day 21 (mg/dL)
Diabetic control	256.75 ± 56.56	254.00 ± 41.86	247.50 ± 41.87	258.25 ± 56.59
125 mg/kg AD	241.50 ± 21.68	224.25 ± 16.91	204.50 ± 13.56*	181.25 ± 12.28**
250 mg/kg AD	196.25 ± 15.33	172.50 ± 14.55	145.75 ± 11.65*	102.25 ± 7.59***
500 mg/kg AD	185.75 ± 8.52	180.75 ± 11.62	133.75 ± 5.66**	83.00 ± 3.92***
Glibenclamide	208.00 ± 23.50	159.75 ± 20.80*	120.00 ± 12.06**	73.25 ± 7.64***
Negative control	70.68 ± 3.10***	73.78 ± 2.50***	79.30 ± 2.30***	75.65 ± 3.43***

Values are mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 vs. diabetic control. One-way ANOVA followed by Tukey's multiple comparison test. AD: *Anchomanes difformis*

In respect of the liver, AD at 500 mg/kg dose elicited significant elevation in the levels of SOD (*P<0.05*) and CAT (*P<0.001*) versus diabetic control, and non-significant diminution in MDA level (*P>0.05*) in diabetic rats. *A. differmis* at 125 and 250 mg/kg elicited a non-significant change in the levels of GSH, SOD, CAT and MDA. Glibenclamide, however, elicited notable increase in the levels of SOD (*P<0.05*) and CAT (*P<0.001*), non-significant change in GSH level and significant diminution (*P<0.05*) in MDA level versus diabetic control (Table 9). Concerning the kidneys, AD at 500 mg/kg dose elicited significant elevation in the levels of SOD (*P<0.00l*) and CAT (*P<0.01*) versus diabetic control, with non-significant change (*P*>0.05) in GSH and MDA levels in diabetic rats. *A. differmis* at 125 and 250 mg/kg elicited a non-significant change in the levels of GSH, SOD, CAT and MDA. Glibenclamide, like AD 500 mg/kg, elicited notable increase in the levels of SOD (*P<0.05*) and CAT (*P<0.001*) versus diabetic control, with non-significant change (*P>0.05*) in GSH and MDA (Table 10). In respect of the pancreas, AD at 500 mg/kg dose elicited significant elevation in the levels of SOD (*P<0.01*) and CAT (*P<0.05*) versus diabetic control, with non-significant change (*P>0.05*) in GSH and MDA levels in diabetic rats. *A. differmis* at 125 and 250 mg/kg elicited a non-significant change in the pancreatic levels of GSH, SOD, CAT and MDA. Glibenclamide, like AD 500 mg/kg, elicited notable increase in the pancreatic levels of SOD (*P<0.01*) and CAT (*P<0.05*) versus diabetic control, with non-significant change (*P*>0.05) in GSH level and a notable decrease (*P<0.01*) in MDA level (Table 11).

While there was a non-significant change in the weight of the pancreas in diabetic rats, there was a dose-dependent decrease in the weight of the liver and kidneys across all doses of AD. 500 mg/kg AD elicited a notable decrease in the weight of the liver (*P<0.001*) and kidneys (*P<0.05*) versus diabetic control. 250 mg/kg AD, on the other hand, produced a significant diminution (*P*<0.05) in the weight of the kidneys. Like AD 500 mg/kg, glibenclamide elicited a notable decrease in the weight of the liver (*P<0.001*) and kidneys (*P<0.05*) versus diabetic control (Table 12).

In comparison to the negative control (non-diabetic and non-treated animals), animals in the diabetic control group had significant increases in the levels of AST, ALP, creatinine and urea. *A. differmis* at 250 and 500 mg/kg elicited significant diminution in the levels of ALP (*P<0.01*, 250 and 500 mg/kg), creatinine (*P<0.001*, 250 and 500 mg/kg), and urea (*P<0.001*, 250 and 500 mg/kg) versus diabetic control. *A. differmis* at all doses produced significant increase in the levels of total protein (*P<0.01*, 125 mg/kg; *P<0.001*, 250 and 500 mg/kg versus diabetic control respectively). Glibenclamide, on the other hand, elicited significant decrease in the levels AST (*P<0.001*), ALP (*P<0.001*), creatinine (*P<0.001*) and urea (*P<0.001*), and significant elevation in the level of total protein (*P<0.001*) versus diabetic control (Table 13).

A. differmis at all doses elicited a non-significant change in the levels of HDL, LDL, cholesterol and triglycerides, save for AD 500 mg/kg which elicited a significant decrease in the cholesterol level (*P<0.05*) in comparison with the diabetic control group.

Table 8: Effect of *Anchomanes differmis* extract on body weight of diabetic rats

Treatments	Day 1 (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)
Diabetic control	133.70 ± 8.01	127.51 ± 7.24	134.70 ± 7.39	136.00 ± 6.34
125 mg/kg AD	140.80 ± 1.64	142.30 ± 1.27	148.77 ± 0.84	141.70 ± 1.26
250 mg/kg AD	137.30 ± 6.36	132.50 ± 6.14	121.64 ± 7.59	135.55 ± 5.43
500 mg/kg AD	159.70 ± 8.04	153.03 ± 7.71	157.50 ± 6.18	162.29 ± 12.86
Glibenclamide	151.28 ± 1.70	144.50 ± 2.21	149.50 ± 2.35	155.50 ± 1.39
Negative control	133.30 ± 2.03	138.50 ± 2.29	149.20 ± 2.30	167.70 ± 1.23

Values are mean±SEM (n=6). *P*> 0.05. One-way ANOVA followed by Tukey's multiple comparison test.
AD: *Anchomanes differmis.*

Table 9: Effect of *Anchomanes differmis* extract on antioxidant indices and MDA level in the liver of diabetic rats

Treatments	GSH (U/mg pro)	SOD (U/mg pro)	CAT (U/mg pro)	MDA (U/mg pro)
Diabetic control	0.057 ± 0.003	1.38 ± 0.07	7.51 ± 0.11	0.116 ± 0.020
125 mg/kg AD	0.063 ± 0.008	1.88 ± 0.04	8.69 ± 0.21	0.099 ± 0.003
250 mg/kg AD	0.074 ± 0.014	2.14 ± 0.03	6.85 ± 3.39	0.092 ± 0.002
500 mg/kg AD	0.071 ± 0.009	4.11 ± 0.11*	13.93 ± 0.79***	0.081 ± 0.001
Glibenclamide	0.237 ± 0.010	4.24 ± 0.04*	16.61 ± 0.18***	0.051 ± 0.018*
Negative control	0.296 ± 0.010	4.54 ± 0.08*	16.38 ± 0.52***	0.025 ± 0.001

Values are mean±SEM (n=6). $^{*}P$<0.05, $^{***}P$<0.001 vs. diabetic control. One-way ANOVA followed by Tukey's multiple comparison test. AD: *Anchomanes differmis.*

Table 10: Effect of *Anchomanes differmis* extract on antioxidant indices and MDA level in the kidney of diabetic rats

Treatments	GSH (U/mg pro)	SOD (U/mg pro)	CAT (U/mg pro)	MDA (U/mg pro)
Diabetic control	0.185 ± 0.005	2.42 ± 0.07	14.97 ± 1.49	0.180 ± 0.010
125 mg/kg AD	0.153 ± 0.012	2.78 ± 0.35	15.29 ± 1.61	0.090 ± 0.004
250 mg/kg AD	0.157 ± 0.040	4.96 ± 0.23	16.87 ± 3.68	0.060 ± 0.005
500 mg/kg AD	0.170 ± 0.021	6.87 ± 0.77*	21.48 ± 3.18**	0.062 ± 0.001
Glibenclamide	0.190 ± 0.001	6.96 ± 0.67**	25.64 ± 1.42***	0.040 ± 0.001
Negative control	0.392 ± 0.010	6.83 ± 0.74**	28.91 ± 1.30***	0.030 ± 0.002

Values are mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 vs. diabetic control. One-way ANOVA followed by Tukey's multiple comparison test. AD: *Anchomanes differmis.*

On the other hand, glibenclamide elicited significant decrement in the levels of LDL (P<0.05), cholesterol (P<0.05) and triglycerides (P<0.05) versus diabetic control, with a non-significant increase in HDL level (Table 14).

Table 15 shows a synopsis of the findings from the histopathological presentations of vital organs (kidneys, liver, and pancreas) harvested from diabetic rats after sub-acute antidiabetic test. In the liver, the administration of AD 125 mg/kg led to the presence of micro globules of fat in the liver tissues. This is similar to the finding in the diabetic control group in which the hepatocytes contain large globules of fat. Glibenclamide and AD 500 mg/kg showed normal hepatocytes (Figure 1 A-E). In the kidney, AD at all doses, as well as glibenclamide, did not elicit any untoward effect on the histopathologic presentations. Normal kidney cells were observed across all AD and glibenclamide doses (Figure 2 A-E). In respect of the pancreas, the ingestion of AD at all doses did not present with any abnormalities in all the groups, save for the diabetic control group in which there was reduced endocrine component. All other groups had normal islet and acini cells of the pancreas (Figure 3 A-E).

A. differmis across all experimented doses elicited a dose-dependent significant diminution in blood glucose levels from day 14 of administration with 500 mg/kg AD dose producing the most significant blood glucose reduction. The effect observed on day 21 were more prominent than those of day 14 suggesting a gradual build-up of antihyperglycaemic and antidiabetic activity of AD; this has important implication in the potential onset of action and drug design. These observed effects are consistent with the findings of Adeyemi *et al.*[24] and Aderonke *et al.*[13] confirming the antihyperglycaemic effect of AD. Additionally, the blood glucose lowering effects observed with AD were similar to those of glibenclamide under the same experimental conditions. The potential for potentiation of antidiabetic activity when AD and glibenclamide are combined in a diabetic experimental model is worth investigating.

The involvement of oxidative stress in the pathophysiology of DM has been documented with several researches alluding that antioxidant effect is involved in the lowering of blood glucose level in a diabetic disease state[33,46]. In this study, the antioxidant effect of AD was confirmed in the most relevant tissues (liver, kidneys and pancreas) which play key roles in a diabetic disease state. The administration of 500 mg/kg AD produced liver, kidneys and pancreas antioxidant levels in diabetic rats similar to levels in normoglycaemic animals. This means that the administration of AD is able to reverse oxidative stress produced by the induction of diabetes in the animals. Furthermore, the tissue antioxidant levels (GSH, SOD, and CAT) produced by AD was similar to those of glibenclamide under the same experimental conditions. Alabi *et al.*[46] confirmed the antioxidant effect of AD extract and proposed that the observed increase in antioxidant indices by AD may be related to increased expression of nuclear factor-erythroid 2-related factor 2 (Nrf2); activation of Nrf2 is one of the pathways initiated during oxidative stress to mitigate the overproduction of free radicals. Alabi *et al.*[46] also opined that the administration of AD reduced pro-inflammatory cytokines, increased anti-inflammatory markers and enhanced antioxidant defence in heart of diabetic animals, thereby making it useful in the prevention and management of associated diabetic complications.

In this study, the administration of AD led to a significant reversal in the levels of ALP with a non-significant reduction in the levels of AST and ALT across AD doses. The significant reduction in the level of ALP may be indicative of a possible hepatoprotective effect of AD at 250 and 500 mg/kg doses. Histopathological assessment of representative liver tissues of diabetic control animals confirmed the presence of congestion and large globules of fat in the hepatocytes. This effect was reversed by the administration of AD at the dose of 500 mg/kg in which the hepatocytes were restored to normal, similar to what was observed with glibenclamide. It is important to add, however, that there was a reduction in the weight of the liver in the 500 mg/kg AD group in comparison with the diabetic control group. It is known that reduction in the weight of vital organs is indicative of toxicity after exposure to toxic substances.[47,48] However, in this study, the induction of DM in animals with nicotinamide-STZ combination led to significant increase in the weight of the liver and kidneys; this is consistent with literature in which DM disease state significantly increased the size of the kidneys and liver.[49] It is therefore possible that AD counteracted the effect of the diabetic state on the weight of the liver towards protection. This is further corroborated with the lack of congestion or histopathological anomalies in the liver after administration of AD. This explanation can be extrapolated to the kidneys which were enlarged after induction of DM and restored after administration of AD. Moreover, no histopathological changes were observed in the kidneys. The pancreatic tissues were unaffected and remained the same as confirmed by the histopathological assessment with no significant change in weight.

Table 11: Effect of *Anchomanes differmis* extract on antioxidant indices and MDA level in the pancreas of diabetic rats

Treatments	GSH (U/mg pro)	SOD (U/mg pro)	CAT (U/mg pro)	MDA (U/mg pro)
Diabetic control	0.060 ± 0.014	1.51 ± 0.12	12.78 ± 1.45	0.053 ± 0.002
125 mg/kg AD	0.070 ± 0.015	2.13 ± 0.24	14.23 ± 1.01	0.051 ± 0.002
250 mg/kg AD	0.060 ± 0.015	2.47 ± 0.59	14.38 ± 0.70	0.046 ± 0.002
500 mg/kg AD	0.100 ± 0.053	3.73 ± 0.13**	15.28 ± 1.63*	0.042 ± 0.002
Glibenclamide	0.135 ± 0.045	3.97 ± 0.42**	15.35 ± 1.49*	0.003 ± 0.005**
Negative control	0.367 ± 0.014	4.06 ± 0.15	17.52 ± 0.38	0.012 ± 0.001

Values are mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01 vs. diabetic control. One-way ANOVA followed by Tukey's multiple comparison test. AD: *Anchomanes differmis.*

Table 12: Effect of *Anchomanes differmis* extract on weight of vital organs of diabetic rats

Treatments	Liver (g)	Kidney (g)	Pancreas (g)
Diabetic control	5.99 ± 0.23	1.10 ± 0.06	0.85 ± 0.03
125 mg/kg AD	5.44 ± 0.11	0.84 ± 0.04	0.82 ± 0.05
250 mg/kg AD	5.02 ± 0.35**	0.58 ± 0.02	0.81 ± 0.03
500 mg/kg AD	4.98 ± 0.27***	0.54 ± 0.03*	0.73 ± 0.02
Glibenclamide	3.76 ± 0.07***	0.50 ± 0.04*	0.80 ± 0.05

Values are mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 vs. diabetic control. One-way ANOVA followed by Tukey's multiple comparison test. AD: *Anchomanes differmis.*

Table 13: Effect of *Anchomanes difformis* extract on biochemical indices in diabetic rats

Group	AST (iu/L)	BIL (mg/dL)	ALT (iu/L)	ALB (mg/L)	ALP (iu/L)	CREAT (mg/dL)	UREA (mg/dL)	TP (mg/L)
Diabetic control	545.10 ± 53.66	0.16 ± 0.10	71.80 ± 3.24	32.53 ± 3.73	417.53 ± 3.54	36.24 ± 1.192	17.63 ± 0.66	61.52 ± 0.75
125 mg/kg AD	507.00 ± 11.25	0.48 ± 0.22	50.07 ± 6.65	35.00 ± 1.63	365.81 ± 2.70	33.47 ± 2.57	13.15 ± 3.32*	66.55 ± 1.00**
250 mg/kg AD	473.00 ± 18.15	0.63 ± 0.20	52.07 ± 1.96	39.85 ± 0.86	266.63 ± 6.43**	25.06 ± 1.07***	7.63 ± 1.84***	71.76 ± 2.63***
500 mg/kg AD	482.00 ± 18.16	1.53 ± 0.38	44.36 ± 2.13	44.10 ± 2.57	253.23 ± 13.02**	22.93 ± 1.87***	7.00 ± 1.30***	78.39 ± 1.38***
Glibenclamide	323.33 ± 50.26***	0.75 ± 0.21	43.52 ± 2.11	43.80 ± 1.64	234.45 ± 2.68***	20.16 ± 0.98***	5.35 ± 0.44***	78.06 ± 0.84***
Negative control	308.25 ± 2.98***	0.31 ± 0.07	30.95 ± 1.45	47.25 ± 2.70	231.5 ± 2.97***	15.38 ± 1.33***	3.68 ± 0.23***	80.01 ± 2.70***

Values are mean±SEM (n=6). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. diabetic control. One-way ANOVA followed by Tukey's multiple comparison test.
AD: *Anchomanes difformis.* AST: Aspartate transaminase. BIL: Bilirubin. ALT: Alanine transaminase. ALB: Albumin. ALP: Alkaline phosphatase. CREAT: Creatinine. TP: Total protein.

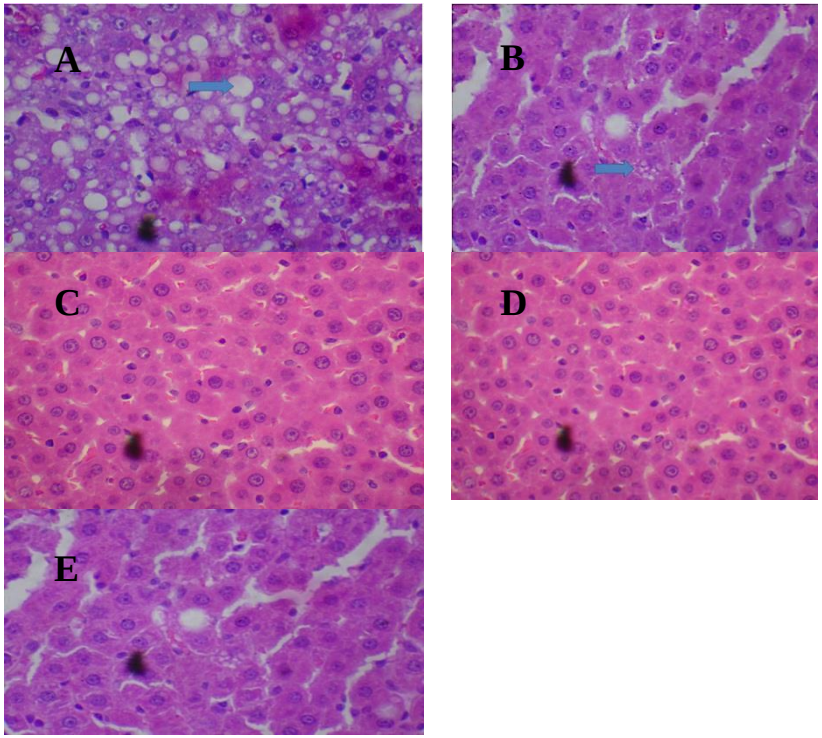

Figure 1: Histopathological presentation of liver tissue from diabetic rats - A. Control group (congested); B. AD 125 mg/kg group (congested); C. AD 250 mg/kg group (normal); D. AD 500 mg/kg group (normal); E. Glibenclamide 2.5 mg/kg (normal) (×400).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 14: Effect of *Anchomanes difformis* extract on lipid profile of diabetic rats

Treatments	HDL (mg/dL)	LDL (mg/dL)	CHOL (mg/dL)	TRIG (mg/dL)
Diabetic control	1.873 ± 0.079	0.523 ± 0.057	2.163 ± 0.064	1.506 ± 0.227
125 mg/kg AD	1.885 ± 0.032	0.457 ± 0.041	1.625 ± 0.101	1.4775 ± 0.087
250 mg/kg AD	1.937 ± 2.193	0.475 ± 0.227	1.115 ± 0.773	1.381 ± 0.433
500 mg/kg AD	1.993 ± 0.133	0.326 ± 0.018	0.773 ± 0.145*	1.430 ± 0.043
Glibenclamide	2.155 ± 0.064	0.188 ± 0.022*	0.537 ± 0.045*	0.443 ± 0.044*
Negative control	2.335 ± 0.065	0.188 ± 0.015*	0.507 ± 0.065*	0.352 ± 0.025*

Values are mean±SEM (n=6). *P<0.05 vs. diabetic control. One-way ANOVA followed by Tukey's multiple comparison test. AD: *Anchomanes difformis.* HDL: High density lipoproteins. LDL: Low density lipoproteins. CHOL: Cholesterol. TRIG: Triglycerides.

Table 15: Effect of *Anchomanes difformis* extract on histopathological presentations of the liver, kidneys and pancreas.

Group	Liver	Kidneys	Pancreas
Diabetic control	Hepatocytes contain large globules of fat	No obvious pathology seen	Reduced endocrine component (islet)
125 mg/kg AD	Presence of microglobules of fat.	Normal kidney cells	Normal islet and normal acini cells
250 mg/kg AD	No obvious pathology seen	Normal kidney cells	Normal islet and normal acini cells.
500 mg/kg AD	Normal hepatocytes	Normal kidney cells	Normal islet and normal acini cells.
Glibenclamide	Normal hepatocytes	Normal kidney cells	Normal islet and normal acini cells.

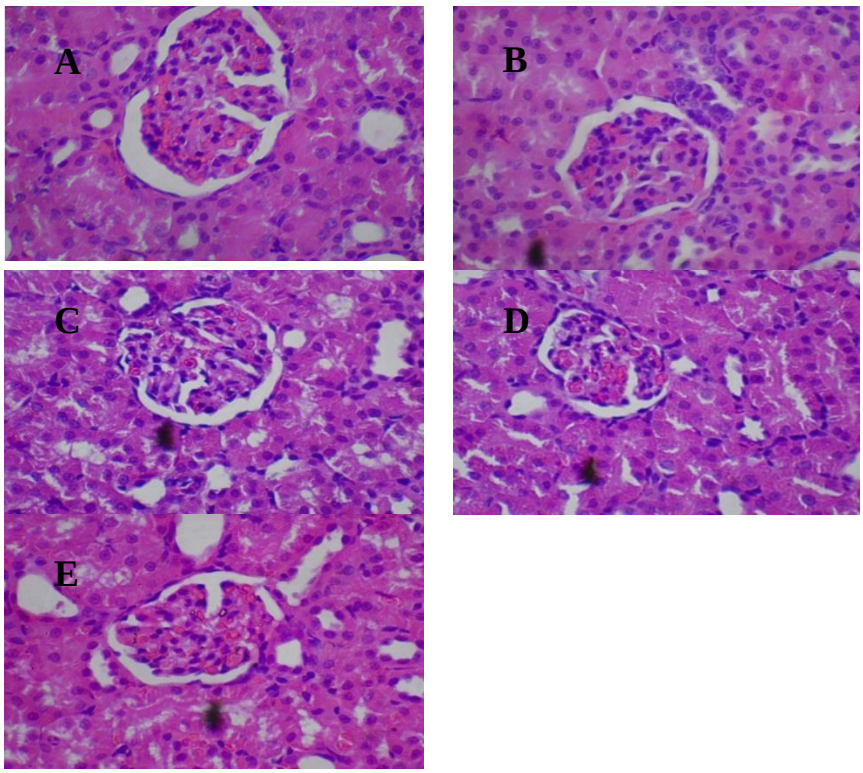

Figure 2: Histopathological presentation of kidney tissue from diabetic rats - A. Control group (normal); B. AD 125 mg/kg group (normal); C. AD 250 mg/kg group (normal); D. AD 500 mg/kg group (normal); E. Glibenclamide 2.5 mg/kg (normal) (×400).

Consistent with the findings of Adeyemi *et al.*,[24] the administration of AD to diabetic rats led to significant decrease in the levels of urea and creatinine, and significant increase in the level of total protein in diabetic rats. An increase in serum urea and creatinine levels in diabetic rats may indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine. Based on findings from this study, AD may have enhanced the ability of the kidneys to remove these waste products from the blood as indicated by the reduction in serum urea and creatinine levels; thus, confer a protective effect on the kidneys of diabetic rats. Taken together, it is possible to suggest that AD extract might directly improve the structural and functional integrities of cells of the liver and kidney. This is, however, inconsistent with the findings of Ataman and Idu[50] which suggests that there is a dose-dependent renal toxicity associated with the administration of AD, although in a non-diabetic state.

Lastly, in this present study, AD at the dose of 500 mg/kg significantly reduced cholesterol levels in diabetic rats. This is consistent with findings from Adeyemi *et al.*[24] in which DM led to an increase in the

level of cholesterol which was reversed by administration of AD. In this present study, AD produced a non-significant dose-dependent reduction in the levels of LDL and triglycerides, with a non-significant dose-dependent increase in HDL. All of these effects suggest the potential of AD to improve lipid profile in the diabetic animals.

Conclusion

From the findings in this study, it can be concluded that the hydroethanolic extract of the rhizomes of *Anchomanes difformis* (Blume) possesses significant antihyperglycaemic and antidiabetic effect and maybe useful in the management of diabetes mellitus. Findings from this study show that the extract of *A. difformis* may not be hypoglycaemic in nature and works best as an antihyperglycaemic in the presence of diabetes mellitus disease state. This present study suggests the *in vivo* antioxidant, hepatoprotective, reno-protective and lipid profile improvement potential of *A. difformis*. However, more research is recommended to confirm these findings.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors express gratitude to Mr. Duncan Ota (Department of Physiology), Mr. Sunday Adenekan (Department of Biochemistry), and Mr. Daniel Osiagwu (Department of Anatomic and Molecular Pathology) of the Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria for the technical assistance rendered in the course of this study.

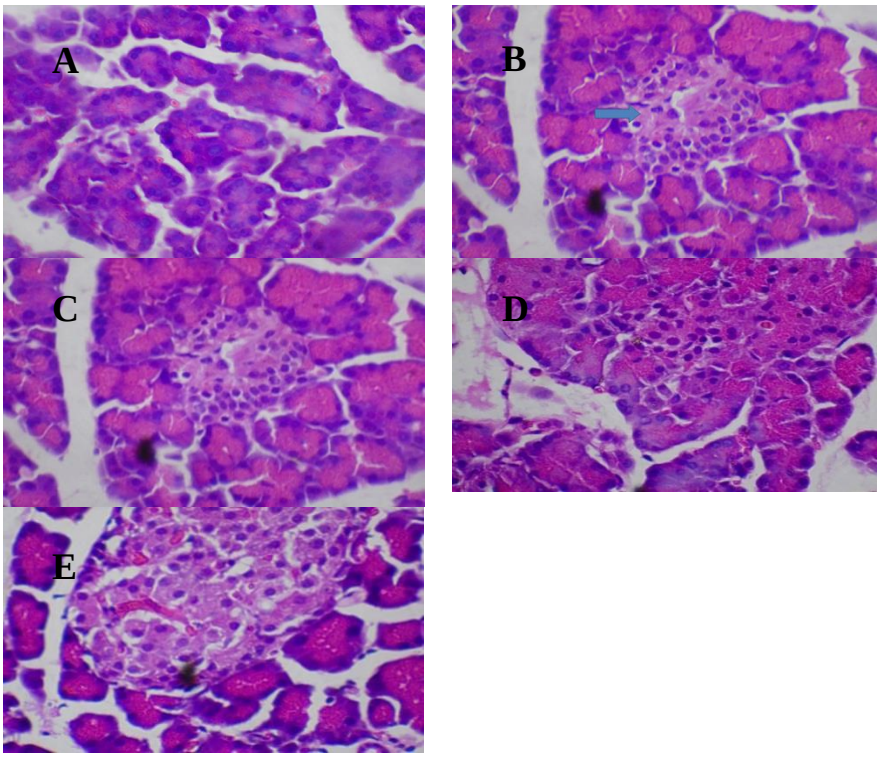

Figure 3: Histopathological presentation of pancreatic tissue from diabetic rats - A. Control group (congestion); B. AD 125 mg/kg group (normal); C. AD 250 mg/kg group (normal); D. AD 500 mg/kg group (normal); E. Glibenclamide 2.5 mg/kg (normal) (×400).

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