Original Research Article

HAEMATOLOGICAL AND HYPOLIPIDEMIC EFFECTS OF METHANOL EXTRACT OF *Annona muricata* (Annonaceae) SEEDS IN ALLOXAN-INDUCED DIABETIC ALBINO RATS

ABSTRACT

This study investigated the haematological and hypolipidemic effects of *Annona muricata* seed methanol extract in alloxan-induced diabetic rats. Thirty healthy male albino rats were divided into five groups. Group 1 served as the normal control; group 2 served as the negative control (induced by alloxan at 125 mg/kg body weight); group 3 served as the positive control treated with glibenclamide (5 mg/kg body weight); and groups 4 and 5 served as the diabetic control group (treated with 50 and 100 mg/kg body weight of *Annona muricata* seed methanol extract, respectively). The diabetic control, diabetic Glibenclamide, and the treated groups were induced with diabetes by intraperitoneal injection of 125 mg/kg bodyweight alloxan monohydrate, and confirmation was done using a glucometer. Treatment lasted for 28 days, after which rats were sacrificed and blood samples were collected through a cardiac puncture for biochemical analysis using standard techniques (Randox kits). Our findings showed a significant increase ($p > 0.05$) in white blood cells (WBC), haemoglobin (Hb), red blood cells (RBC), and packed cell volume (PCV) of the extract when compared with the non-treated group (negative control). The graded doses of the extract significantly ($p <0.05$) increased high-density lipoprotein (HDL), while total cholesterol (TC), triacylglycerides (TG), and low-density lipoprotein (LDL) were significantly ($p <0.05$) reduced compared to the diabetic untreated group. The findings imply that *Annona muricata* seed methanol extract is risk-free, effective at treating some biochemical and haematological abnormalities linked to diabetes mellitus, and might be suggested as a supplement to dietary therapy.

Keywords: Alloxan, Annona muricata, Diabetes, Haematology, Hypolipidemic

INTRODUCTION

Diabetes mellitus is a metabolic condition characterized by disturbance in the breaking down of carbohydrates, lipids and proteins due to moderate or average insulin deficiency [1,2]. Diabetes is a symptom disease with a peculiar feature of high blood glucose levels due to imbalanced insulin production [1]. Through atherogenic dyslipidemia, which is characterized by an increase in total cholesterol, triglycerides, low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL), and a decrease in high-density lipoproteins

(HDL) particles, diabetes is linked to a higher risk of death from cardiovascular disease (CVD)[3,4].Additionally, it has been hypothesized that anaemia in diabetes mellitus results from an increase in non-enzymatic glycosylation of red blood cells (RBC) membrane proteins, which is correlated with hyperglycemia [5]. All diabetes mellitus treatment plans include lowering blood glucose levels to those in the normal range while reducing cardiovascular risk, especially controlling hypertension and correcting dyslipidemia [6]**.** Several medicinal plants are utilized as traditional treatments for diabetes because they are efficient, have fewer side effects, and are reasonably inexpensive [2]**.** One of these herbs, *Annona muricata* (Annonaceae), is utilized in Nigerian folk medicine to treat diabetes mellitus.

Annona muricata (Sour-sop) seeds are nutrient-dense and provide a good amount of acetogenins-containing compounds, namely bulatacin, asimisin and squamosin [7]. Phytochemical analysis of *Annona muricata* leaf extract revealed the presence of some secondary metabolites like tannins, steroids, and cardiac glycosides [8]. Luteolin, quercetin, and tangeretin are only a few of the plant substances found in soursop leaf that have antioxidant properties. The minerals in sour-sop leaves are thought to lower blood sugar levels to a normal range, which is particularly beneficial for managing and treating diabetes [8]. The anti-inflammatory [9], Anti-Arthritic [10], Antidiabetic [11], and anti-cancer [12] properties of *Annona muricata*plant parts have also been highlighted in several studies.

This study investigated the haematological and hypolipidemic effects of *Annona muricata* seed methanol on induced diabetic rats. Specifically, the study sort to determine the changes in haematological parameters (PVC, RBC, and WBC) in induced diabetic rats and investigate the effect of the extract on the lipid parameters (HDL, TCHOL, TAG and LDL) in the experimental rats. This work is important because biochemical and haematological changes are major observable clinical and pathological features common with diabetes. Development of complications is certain in diabetes with increased risk of biochemical and haematological derangement of the markers, hence the relevance of this investigation.

2. MATERIALS AND METHODS

Chemical and Reagents

Alloxan monohydrate (Sigma Aldrich Chemicals, USA) induced diabetes. All other chemicals and reagents used were of analytical grades and products

Plant Materials/Extraction

*Annona muricata*fruits were bought from Orie-Ugba market in Umuahia North LGA, Abia State.The plant was authenticated by a Taxonomist (Dr Ibe K. Ndukwe) from the forestry department, College of Natural Resources and Environment Management (CNREM), Michael Okpara University of Agriculture Umudike (Specimen voucher number = IHF 26125). The seeds were collected, washed,and oven-dried. It was weighed and milled to fine powder 250g. The powered form was soaked in methanol and distilled water in the ratio of 80:20, respectively and was allowed to rest for 3 days with occasional shaking. This was filtered using Whatman No. 1 filter paper. The filtrate was evaporated to obtain the dry matter using a rotary evaporator under reduced pressure at 40^0 C.

Experimental animals

Thirty (30) healthy male Wistar rats weighing 100-120g, obtained from Ogive Integrated farmhouse, Aba, Abia State, were used for the study. The animals, on arrival, were weighed to obtain initial weight and were acclimatized for 14 days in the animal house of the Biochemistry Department, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike.The animals were brought to daylight for 12 hours under normal tropical weather conditions with access to standard food and water till the end of the research, which lasted for 28 days. Throughout the experiment, all the rats were housed at 25^oC in clean metal cages under normal daylight humid conditions. The rats were freely fed pellets, given tap water, and made available throughout the experiment as approved by the departmental committee on animal use guidelines, Michael Okpara University of Agriculture, Umudike on handling experimental animals.

Induction of Diabetes

At the end of acclimatization, the animals were allowed to fast and then diabetes was induced by intra-peritoneal (IP) injection of 120mg/kg body weight of alloxan monohydrate solution. Animals with blood glucose level higher than 150mg/dl were considered diabetic after 3 days of induction using fasting blood sugar method and were selected for the study.

Experimental Design and Animal Grouping

Rats were divided into five groups of six rats each: Group 1 (Normal control), group 2 (Negative control), group 3 (Positive/Glibenclamide), group 4 (animals treated with 50mg/kg of AMSE extract), group 5 (animals treated with 100mg/kg of AMSE extract), respectively.

Groups		Treatment
Group 1	Normal control	Feed + H_2O ad libitum
Group 2	Negative control	Alloxan + Feed + H_2O ad libitum
Group 3	Positive control	Alloxan+ Standard drug (Glibenclamide) + Feed + H_2O
Group 4	*Annona muricata*	Alloxan + 50mg/kg extract + Feed + H_2O ad libitum
Group 5	*Annona muricata*	Alloxan + 100mg/kg extract + Feed + H_2O ad libitum

Sacrifice and Sample Collection

After the experiment, Blood samples were collected through cardiac puncture under anaesthesia into an EDTA bottle. Pooled blood sample (1 ml per rat, 9 ml per treatment) was used for biochemical analysis.

Determination of Biochemical Parameters

Total cholesterol was evaluated using the enzymatic colourimetricchod-pap testmethod[14]; Triglycerides were also determined spectrophotometrically using the method of Tietz [15]; High-density lipoproteins (HDL) were evaluated by the method of Grove [16]. Low-density lipoprotein (LDL) was determined as the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethene-glycol monomethyl ether [17].

Determination of Haematological Parameters

Haematological parameters were analyzed using a haematology analyzer (Mindray Auto Hematology Analyzer, BC-5200, USA.) following the methods of Chhabra [18]. The parameters assayed were as follows: white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), and packed cell volume (PCV)

Statistical Analysis

Statistical data analysis was carried out with SPSS version 22.0 using One Way Analysis of variance (ANOVA). The statistical analysis data were reported as Mean ± standard deviation (SD). A significant difference using Tukey's Post Hoc test was accepted at 95% confidence level of probability, i.e., if $p<0.05$

3.0 RESULT

Table1: Weight of Diabetic rats treated with methanol extract of Sour-sop (*Annona muricata)* seed

Our findings indicated a relatively increased weight in rats administered 50 mg/Kg and 100 mg/Kg AMSE extract, respectively, at weeks 1, 2 and 3 compared to week 0.

Groups	Treatment	week 0 (g)	week 1 (g)	week 2 (g)	Week 2 (g/dl)
1	Normal Control (Feed + H_2O ad libitium)	112.13 ± 0.230	105.14 ± 1.20*	114.24 ± 2.20*	135.4 ± 2.705*
2	Negative Control (Alloxan + Feed + H_2O ad libitium)	119.42 ± 4.404	121.27 ± 2.170*	127.63 ± 2.430*	131.8 ± 6.720*
3	Positive Control (Alloxan + Standard drug, Glibenclamide + Feed + H_2O ad libitium)	109.20 ± 1.610	113.45 ± 3.601*	123.04 ± 1.366*	127.7 ± 2.370*

4	AMSE 50mg/kg extract (Alloxan + Feed + H_2O ad libitium)	100.14 ± 3.064	118.34 ± 2.500*	124.57 ± 0.740*	129.4 ± 0.620*
5	AMSE100mg/kg extract (Alloxan + Feed + H_2O ad libitium)	105.25 ± 5.301	125.34 ± 1.405*	131.41 ± 0.401*	133.5 ± 0.670*

The table is expressed as mean ± SEM* n=5, $p<0.05$ significant difference compared to week 0.
AMSE: *Annona muricata* seed methanol extract

Table2: Effect of Haematological indices of alloxan-induced diabetic albino rats treated with methanol extract of Sour-sop (*Annona muricata)* seed

Our findings showed a significant (P< 0.05) reduction in TWBC, HB, RBC and PCV in the diabetic animals. Treatment with AMSE extracts significantly (P< 0.05) improves the levels of these indices in diabetic animals.

Groups	Treatment	RBC (g/dl)	TWBC (g/dl)	Hb (g/dl)	PCV (g/dl)
1	Normal Control (Feed + H_2O ad libitium)	165.13 ± 0.30	75.14 ± 1.20	12.24 ± 2.20	53.24 ± 2.05
2	Negative Control (Alloxan + Feed + H_2O ad libitium)	131.32 ± 1.40	43.20 ± 2.10	8.13 ± 2.00	31.16 ± 1.20
3	Positive Control (Alloxan + Standard drug, Glibenclamide + Feed + H_2O ad libitium)	160.20 ± 1.10*	73.05 ± 3.01*	11.04 ± 1.30*	53.07 ± 2.30*
4	AMSE50mg/kg extract (Alloxan + Feed + H_2O ad libitium)	170.14 ± 3.04*	78.04 ± 2.00*	14.17 ± 0.40*	55.04 ± 0.20*
5	AMSE100mg/kg extract (Alloxan + Feed + H_2O ad libitium)	173.25 ± 5.01*	82.34 ± 1.05*	15.01 ± 0.01*	58.24 ± 0.10*

The table is expressed as mean ± SEM*, p<0.05 significant difference compared to the diabetic untreated (group 2). Values are expressed as mean ± SD (n = 5). *p<0.05 when compared with the negative control.

Abbreviation: TWBC: Total White Blood Cells; Hb: Hemoglobin; RBC: Red Blood Cells; PCV: Packed Cell Volume. AMSE: *Annona muricata* seed methanol extract

Table 3: Effect of Lipid profile of alloxan-induced diabetic albino rats treated with methanol extract of Sour-sop (*Annona muricata)* seed

The triacylglycerol level in the diabetic control animals was significantly ($p < 0.05$) higher than that of the healthy animals. Treatment with AMSE in all the administered doses significantly lowered triacylglycerol levels in the diabetic animal to levels comparable to that of the normal control animals. This was similar to the effect of Glibenclamide, which was also able to lower significantly ($p < 0.05$) the triacylglycerol level in diabetic animals. Our results also showed a significant elevation in the level of cholesterol in the diabetic animal compared to the healthy control animals. The AMSE - treated group showed a significant ($p < 0.05$) decrease in cholesterol level compared to the diabetic control animals. Glibenclamide also significantly ($p < 0.05$) decreased cholesterol levels in diabetic animals. The concentration of HDL in the diabetic animals was significantly ($p < 0.05$) lower than that of the healthy animals. The different doses of AMSE (50 and 100 mg/kg) were able to significantly ($p < 0.05$, respectively) elevate the reduced HDL in the diabetic animals. Glibenclamide also significantly ($p < 0.05$) elevated HDL levels in diabetic animals. Induction of diabetes elevated the LDL levels of the animals. However, AMSE at different doses (50 and 100 mg/kg) were able to significantly ($p < 0.05$, respectively) elevate the reduced LDL in the diabetic animals

Groups	Treatment	TCHOL (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
1	Normal Control (Feed + H_2O ad libitium)	75.12 ± 1.230	72.14 ± 4.420	15.24 ± 10.20	83.04 ± 2.105
2	Negative Control (Alloxan + Feed + H_2O ad libitium)	91.02 ± 1.450	163.20 ± 3.170	78.13 ± 2.300	31.16 ± 1.720
3	Positive Control (Alloxan + Standard drug, Glibenclamide + Feed + H_2O ad libitium)	66.21 ± 1.140	90.25 ± 30.31*	13.04 ± 1.430*	73.27± 2.430*
4	AMSE 50mg/kg extract (Alloxan + Feed + H_2O ad libitium)	70.17 ± 3.724	101.4 ± 2.030*	24.17 ± 0.440*	65.84 ± 6.720*
5	AMSE 100mg/kg extract (Alloxan + Feed + H_2O ad libitium)	57.25 ± 2.051	92.34 ± 1.405*	10.41 ± 10.01*	68.24 ± 5.310*

The table is expressed as mean ± SEM* n=5, $p<0.05$ significant difference compared to the diabetic untreated (group 2). **Abbreviation:** TCHOL: Total Cholesterol, TG: Triacyl glyceride, HDL: High-density lipoprotein; LDL; Low-density lipoprotein; AMSE: *Annona muricata* seed methanol extract

DISCUSSION

Hyperglycemia and an elevated lipid profile describe diabetes mellitus, a metabolic disease. This condition may be caused by insulin's failure to initiate the cellular absorption of glucose after digestion. However, it is crucial to create more potent and affordable medications to treat and control the condition, given the rise in mortality caused by it. Numerous studies have documented a strong effect of *A. muricata* on variables related to the development of diabetes mellitus. According to studies, antihyperglycemic effects, a rise in body weight, and improved serum lipid profile by lowering TCHO, TRIG and LDL, VLDL, and increasing TCHO, HDL, and the percentage of the anti-atherogenic index (AAI), are all recorded [3].

The most frequent consequences of diabetes mellitus are changes in lipid metabolism, which present as hyperlipidemia. According to studies, lipid profile changes in diabetes patients are a risk factor for cardiovascular illnesses [6]. Compared to the control rat in the research, the alloxan-induced diabetic rats showed hypertriglyceridemia, decreased HDL levels, hypercholesterolemia, and a modest rise in LDL levels. In addition, Alaebo et al. [4] observed that alloxan-induced diabetic rats had higher plasma cholesterol, TAG, LDL-c, VLDL-c, and lower HDL cholesterol. The elevated levels of triglycerides and cholesterol seen in this study may be caused by hormone-sensitive lipase being activated due to insulin insufficiency or sensitivity, which causes a rise in the mobilization of free fatty acids from peripheral stores. MEAM therapy improved HDL levels and decreased triglyceride, cholesterol and LDL levels in diabetic rats. AMSE contains a phenolic substance that contributes to the normalization of the lipid profile, which may explain its capacity to alleviate the changes in lipid metabolism in diabetic animals.

Erythrocytes have been shown to play a very significant role in delivering oxygen to body tissues throughout systemic circulation [19]. Hyperglycemia in diabetes mellitus has been demonstrated to impair the deformability of red blood cells, which are otherwise deformed without rupturing as they withstand continuous flow conditions through narrow capillaries [20]. This impairment is a result of the generation of reactive oxygen species that is observed in diabetes mellitus. This is in line with the study's conclusion that diabetic animals had significantly lower levels of TWBC, HB, RBC, and PCV. Alloxan administration may have decreased WBC, Hb, RBC, and PCV levels because of aberrant haemoglobin synthesis, poor blood osmoregulation, and high plasma osmolarity [20]. As the extract was administered, the RBC level and associated indices significantly improved. This supports the claim that the AMSE extract can promote the production or release of erythropoietin, which prompts bone marrow stem cells to create red blood cells [21].

CONCLUSION

At dosages of 50 and 100 mg/Kg body weight, the ingestion of AMSE extracts has been proven to produce hypolipidemic effects. In this study, the ability to lower blood cholesterol, which may be connected to a high concentration of phytonutrients, was associated with the reversal of the effects of diabetes on numerous biochemical and haematological parameters. Therefore, it can be said that AMSE extracts normalize the haematological anomalies connected to diabetes mellitus

securely and efficiently. They may thus be recommended as an addition to dietary treatment for diabetes.

ETHICAL APPROVAL

All authors with this declare that principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the College of Natural Sciences, Michael Okpara University of Agriculture (MOUAU) Research and Ethics Committee.

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