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HYPOGLYCAEMIC AND HEPATO-PROTECTIVE EFFECTS OF CRUDE EXTRACT OF *AVERRHOA CARAMBOLA* LEAVES IN ALLOXAN-INDUCED FEMALE DIABETIC RATS

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ABSTRACT

Following the increasing rate of use of natural products in management of diabetes mellitus, the hypoglycemic and hepatoprotective effects of methanol extract of *Averrhoa carambola* (MEAC) leaves were investigated in alloxan-induced diabetic albino rats. Thirty (30) albino rats (95–125 g) divided into 5 groups (n = 6) were used. The induction of diabetes was done by intra-peritoneal (IP) injection of 120 mg/kg of alloxan monohydrate. Groups 1-3 served as normal, negative (untreated) and positive (standard drug) controls respectively, while Groups 4-5 were alloxan-induced rats treated with 100, 200 and 400 mg/kg b.w of MEAC respectively. Administration was done orally for twenty-eight (28) days and fasting blood glucose level was obtained at a 7-day interval. After treatment, the rats were anaesthetized and blood collected by cardiac puncture for determination of hepatic and renal indices using standard analytical procedures. The acute toxicity result of MAEC indicated no mortality or adverse reactions up to 5000 mg/kg b.w. MEAC significantly ($p<0.05$) reversed the elevated blood glucose level in the diabetic animals in a time-dependent manner. The elevated hepatic (AST, ALT and ALP) and renal (urea) function indices in the diabetic animals were significantly ($p<0.05$) reduced in the MEAC-treated animals, while albumin concentration was significantly ($p<0.05$) elevated when compared to the negative control. The findings of this study suggest a hypoglycemic effect of

MEAC possibly via the down regulation of blood sugar level. MEAC also showed protective effect against hepatotoxicity and diabetic nephropathy.

Keywords: Alloxan diabetes, Diabetes, Kidney function, Liver profile, , Rats

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by disturbances in the metabolism of carbohydrates, proteins and lipids as a result of absolute or moderate insulin deficiency [1]. Diabetes mellitus is not a single disease instead it is a syndrome having a peculiar feature of raised blood glucose level primarily due to unbalanced insulin production, secretion or action [2]. The World Health Organization has given a report on the global burden of the disease. According to its findings the predominance of the disease in adults is assessed to be about 387 million [3]. In the absence of insulin, the liver increases glucose uptake, but kidney being insulin-independent organ, tries to cope with the higher level of glucose in case of diabetes [4].

Currently available synthetic anti-diabetic agents besides being expensive, produce serious side effects. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes mellitus.Medicinal plants have the advantage of having no side effects [5].

Recently, the association between prevalence of type 2 DM and liver diseases was established. Type 2 DM is a risk factor for progressive liver disease and liver-related death [6]. Adults with newly diagnosed diabetes appeared to be at higher risk of advanced liver diseases. Such diabetes is known as hepatogenous diabetes (HD) [7] and said to be associated with diabetic hepatopathy. In the presence of hepatic disease, the metabolic homeostasis of glucose is impaired because of disorders such as insulin resistance, glucose intolerance and impaired sensitivity of islet β-cells in the pancreas [8]. Therefore, treatment of diabetes in presence of the liver cirrhotic patient is complex. The presence metabolic changes due to liver damage and the hepatotoxicity of oral hypoglycemic drugs further complicate the matter. Therefore, pharmacological therapy must be closely monitored for the risk of hypoglycemia [9].

Averrhoa carambola, belonging to the Oxalidaceae family, is well known as Star fruit or Carambola in Asia, or kpakpando mkpuru in Igbo. It is an age old plant with many medicinal uses and also contains secondary metabolites which have various biological activities [10]. Parts of *A. carambola* have been found useful for therapeutic purposes. A decoction of *carambola* leaves has been reported to be of use in treatment of diabetes [11]. An alcoholic extract of the stems of *A. carambola* has been shown to exhibit selective activity against brain tumor cells while that of the leaves was effective against liver carcinoma cells [12]. The aqueous leaf extract of *A. carambola* depresses atrial inotropism in the guinea pig [13]. Furthermore, several studies have highlighted the anti-inflammatory [14], analgesic [15], hypoglycemic [16]; [17], anthelmintic [18], anti-ulcer [19], hypotensive [20], antioxidant [21], hypocholesterolaemic and hypolipidemic, [17], antimicrobial [22, 14] and antitumor [12] activities of *A. carambola* plant parts. These properties are elicited as a result of presence of important phytochemicals like saponins, alkaloids, flavonoids and tanins [23, 24].

Due to the numerous medical and pharmacological properties of *Averrhoa carambola* for the management of ailments, this study was at aimed at evaluating the possible hypoglycaemic and hepatoprotective effects of methanol extract of *Averrhoa carambola* leaves on alloxan-induced female diabetic rats.

MATERIALS AND METHODS

Chemicals and reagents

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

Plant material

Fresh leaves of *Averrhoa carambola* were plucked from a healthy tree at Lodu, Ndume Ahieke, Umuahia North Local Government Area of Abia State, Nigeria in June 2019. The leaves were authenticated by a Taxonomist (Dr. Ibe K. Ndukwe) in the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Experimental animals

Thirty healthy female albino rats (95–125g) were used for this in the Animal House of Biochemistry Department, Michael Okpara University of Agriculture, Umudike, Abia State. They were housed in aluminum cages in clean conditions and fed standard rat feed and water *ad libitum*. A period of 7 days was allowed for acclimatization before commencement of the experiment. All processes and procedures in handling the rats were in compliance with the guidelines of the National Institute of Health [16].

Preparation of Plant extract

The leaves were peeled, washed with distilled water and then air-dried. After one week, the leaves were pulverized to a fine powder using a mechanical grinder. The ground sample of *Averrhoa carambola* was extracted with 80% Methanol and 20% distilled water for 3 days with intermittent shaking. The solution was properly filtered with a muslin cloth and Whatman filter paper. The solvent was evaporated from the filtrate rate with the help of an electric oven at 40 ^{o}C.

Acute toxicity (LD_{50}) of plant

The acute toxicity test on the leaves was conducted in two phases in accord with Lorke's method [25].

Induction of Diabetes Mellitus

The baseline blood glucose levels of the rats were determined prior to induction of diabetes by intra-peritoneal (IP) injection of 120 mg/kg body weight of alloxan monohydrate solution by the method of Yanardağ and Çolak [26]. Confirmatory test was carried out after three days to confirm diabetic condition.

Experimental Design

The animals were divided randomly into 6 different groups of 5 rats per cage and treated as follows:

Group 1 – (Normal control) receiving only normal saline

Group 2 – (Negative control) induced with diabetes and left untreated

Group 3 – (Positive control) induced with diabetes and treated with standard drug (Glibenclamide, 2 mg/kg bw)

Group 4 – Induced with diabetes and treated with 100 mg/kg b.w of MEAC

Group 5 – Induced with diabetes and treated with 200 mg/kg b.w of MEAC

Group 6 – Induced with diabetes and treated with 400 mg/kg b.w of MEAC

The administration of MEAC was done orally and the blood glucose and body weights of the animals were checked at a 7-day interval during twenty-eight days treatment period. All groups had access to normal feed and water *ad libitum.*

Weight Determination

The weight determination was done using an electronic weighing balance. The weights or the rats were taken in the morning before feeding.

Estimation of blood glucose level

The blood glucose levels of the animals were determined using a Glucometer Accu-check (Tyson Bio Evolve glucometer, Tyson Bioresearch Inc., Hangzhou, China) and subsequently on a weekly basis at days 0, 7, 14, 21 and 28 throughout the period of treatment with standard drug and extract.

Sacrifice of animals and collection of blood samples

After the experiment, the animals were sacrificed under mild anaesthesia with chloroform. Blood samples were obtained into lithium heparin bottles for biochemical analysis.

Hepatic indices

Serum AST and ALT activities were estimated by the method of Reitman and Frankel [27] as outlined in Randox test kit. The activity of alkaline phosphatase (ALP) was assayed using the method of Kochmar and Moss [28]. The concentration of bilirubin was determined using the method of Jendrassik and Grof [29] as outlined in Randox test kits. Total protein estimation was determined by the method of Tietz [30] as stated in Randox test kits.

Renal indices assay

Determination of serum creatinine concentration

Creatinine concentration was determined using Direct Endpoint method according to Henry [31], while urea concentration was determined using Urease-Berthelot method by Fawcett and Scott, [32] both provided by Randox Diagnostics Ltd. UK.

Phytochemical Analysis: Phytochemical tests were carried out on the aqueous, ethanol methanol and n-hexane extracts using standard phytochemical methods as described by Sofawara (1993). The phytochemicals that were assayed include alkaloids, flavonoids, saponins, tannins and phenols.

Statistical analysis

Statistical analysis of the data was carried out with SPSS version 22.0 using One Way Analysis of Variance (ANOVA). The statistically analyzed data were reported as Mean + standard deviation (SD). Significant difference using Tukey's Post Hoc test was accepted at 95% confidence level of probability i.e. if $P< 0.05$.

RESULTSAND DISCUSSION

Changes in bodyweight

Table 1 showed the effect of three doses of star fruit leaves' extracts (100, 200 and 400 mg/kg) and Glibenclimide (2 mg/kg) on the weight of rats. There was a significant ($P<0.05$) increase in weight of the diabetic-induced rats but administration of the extract 200 mg/kg and 400 mg/kg effectively reduced the weights in a dose dependent fashion similar to standard drug showing

significant (P<0.05) decrease in weight when compared to that of the normal and negative controls. A very slight increase (p>0.05) in weight was noticed in the 100 mg/kg extract group similar to the negative control group.

Table 1: Effect of Methanol extract of *Averrhoa carambola* (star fruit) leaves on the mean body weight changes in alloxan-induced test groups and normal rats

Group/Treatment	Week 0	Day 7	Day 14	Day 21
Group I (Normal Control)	112.00 ± 5.78	115.08 ± 6.02	125.53 ± 7.119	134.30 ± 7.20
Group 2 (Negative control)	97.13 ± 9.73	97.63 ± 11.88	103.95 ± 2.76	104.00 ± 2.83
Group 3 (Positive control) 2mg/kg Glibenclamide)	109.80 ± 10.58	108.38 ±10.86	106.63 ± 13.93	109.00 ± 18.38
Group 4 (DM + 100 mg/kg MEAC)	122.00 ± 12.20	123.65 ± 13.72	126.38 ± 15.86	123.57 ± 17.96
Group 5 (DM + 200 mg/kg MEAC)	93.20 ± 6.51	90.57 ± 5.62	90.23 ± 5.84	89.50 ± 7.79
Group 6 (DM + 400 mg/kg MEAC)	120.20 ± 21.44	121.77 ± 32.07	123.70 ± 38.48	111.00 ± 21.66

Result is expressed as mean ± Standard Deviation (n=5). Values are significantly different at P<0.05 along the columns, ns=not significant.

The effect of methanol extract of *Averrhoa carambola* leaves on glucose levels of alloxan-induced diabetic rats

From the results (Figure 2), at Day 0, there was a significant (P<0.05) increase in the glucose level of negative control (364.10±0.05), positive control (390.10±0.07) and across all extract-treated groups when compared to normal control (62.00±1.22). However, a time-dependent significant (p<0.05) decrease in glucose level was noted for Days 7, 14, 21 and 28 in positive control and Groups 4-6 treated with 100, 200 and 400 mg/kg of MEAC respectively when compared with the untreated negative control. Also, at Day 28, Groups 4 (95.30±0.08) and 5 (132.30±0.08) had significantly (p<0.05) lower glucose levels when compared to the positive

control (190.20±0.13) and Group 6 (200.70±0.20).

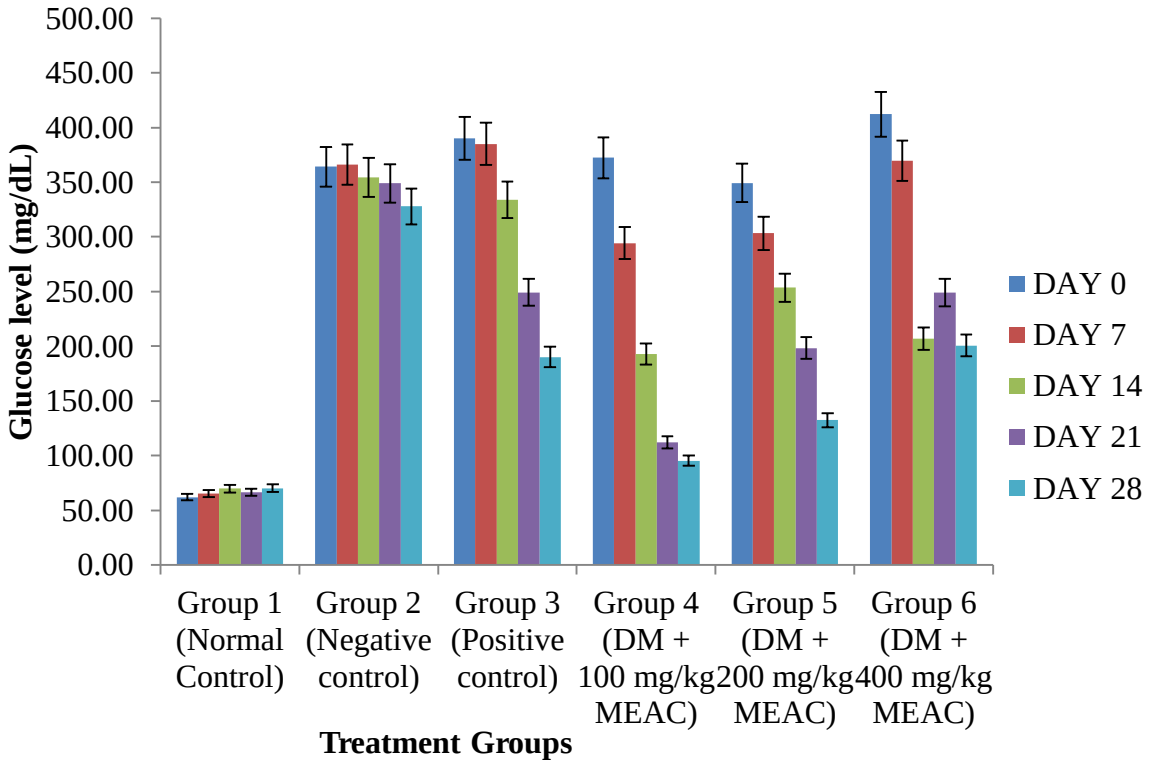

Figure 1: The effect of MEAC on glucose levels of alloxan-induced diabetic rats.
Values are means ± SD (n=5). Bars with different letters are significantly different (P<0.05,).
DM = Diabetes-induced; MEAC = Methanol extract of *Averrhoa carambola.*

Effect of methanol extract of *Averrhoa carambola* leaves on hepatic indices of alloxan-induced diabetic rats

As shown in Table 2, the AST and ALT activities of negative control (81.27±2.78 and 56.60±5.39 respectively) was significantly ($p<0.05$) elevated, whereas, the administration of MEAC in Groups 3-5 and Glibenclamide in positive control caused significant ($p<0.05$) decreases in AST and ALT activities when compared with the negative control. In the same manner, administration of 100 and 200 mg/kg of MEAC caused a significant ($p<0.05$) decrease in ALP activity in Groups 4 (61.78±0.97) and 5 (57.40±0.29) respectively when compared to the negative control (99.82 ± 2.27). Also, the ALP activity in Group 6 (108.79±0.57) was significantly ($p<0.05$) higher when compared to normal control (17.91 ± 0.37), Groups 4

(61.78±0.97) and 5 (57.40±0.29). No significant ($p>0.05$) alteration in total protein and globulin concentrations was observed among all the groups. There was a significant ($p<0.05$) increase in the level of albumin of the positive control (3.00 ±0.04), Groups 3 (3.32±0.19) and 4 (3.05±0.04) compared to the negative control (2.63±0.15).

Table 2: Effects of methanol extract of *Averrhoa carambolla* on hepatic indices of alloxan-induced hyperglycemic rats

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TP (g/dL)	ALB (g/dL)	GLB (g/dL)
Group I (Normal Control)	46.07 ± 6.83*	12.27 ± 0.27*	17.91 ± 0.37*	5.64 ± 0.42	3.41 ± 0.02*	2.22 ± 0.40
Group 2 (Negative control)	81.27 ± 2.78	56.60 ± 5.39	99.82 ± 2.27	5.53 ± 0.42	2.63 ± 0.15	2.90 ± 0.39
Group 3 (Positive control) 2mg/kg Glibenclamide	47.40 ± 2.86*	13.40 ± 1.40*	84.85 ± 7.39*	6.15 ± 0.04	3.00 ± 0.04*	3.15 ± 0.07
Group 4 (DM + 100 mg/kg MEAC)	100.67 ± 2.03*	41.57 ± 1.05*	61.78 ± 0.97*	5.89 ± 0.28	3.32 ± 0.19*	2.57 ± 0.42
Group 5 (DM + 200 mg/kg MEAC)	47.60 ± 3.20*	12.70 ± 0.65*	57.40 ± 0.29*	6.40 ± 0.34	3.05 ± 0.04*	3.35 ± 0.38
Group 6 (DM + 400 mg/kg MEAC)	44.40 ± 5.68*	25.13 ± 0.39*	108.79 ± 0.57*	6.09 ± 0.25	2.86 ± 0.07	3.23 ± 0.30

Results are expressed as Mean ± Standard Deviation (S.D). Mean values with asterisk (*) are significantly different from the negative control group at $P < 0.05$.

Effect of methanol extract of *Averrhoa carambolla* on renal function parameters of alloxan-induced diabetic rats

As shown in **Table 3**, the 100, 200 and 400 mg/kg body weight of MEAC significantly ($p<0.05$) lowered the urea concentrations of the rats in Groups 4 (77.96±0.11 mg/dl), 5 (57.90 ± 0.70 mg/dl) and 6 (54.67 ± 0.64 mg/dl) when compared to negative control group (100.78±1.41 mg/dL). The effect of the extract at the doses of 200 and 300 mg/kg bw were comparable to that of the positive control (57.82±5.85 mg/dl) as there were no significant ($p>0.05$) difference between the urea concentrations in these groups. There was no significant ($p>0.05$) difference in creatinine concentrations of the extract-treated groups when compared to the negative control. However, the creatinine concentration of the normal control (0.57±0.13 mg/dl) was significantly ($p<0.05$) lower than that of negative control group (1.21±0.13 mg/dl).

Table 3: Effect of methanol extract of *Averrhoa carambolla* leaveson renal function parameters of alloxan-induced diabetic rats

Groups	Treatment	Urea (mg/dL)	Creatinine (mg/dL)
1	**Group I (Normal Control)**	27.83 ± 0.72*	0.57 ± 0.13*
2	**Group 2 (Negative control)**	100.78 ± 1.41	1.21 ± 0.13
3	**Group 3 (Positive control) 2mg/kg Glibenclamide**	57.82 ± 5.85*	1.97 ± 0.06*
4	**Group 4 (DM + 100 mg/kg MEAC)**	77.96 ± 0.11*	1.21 ± 0.16
5	**Group 5 (DM + 200 mg/kg MEAC)**	57.90 ± 0.70*	1.08 ± 0.13
6	**Group 6 (DM + 400 mg/kg MEAC)**	54.67 ± 0.64*	1.19 ± 0.02

Results are expressed as Mean ± Standard Deviation (SD). Mean values with asterisk (*) are significantly different from the negative control group at $P < 0.05$.

Clinical and experimental evidence suggests that diabetes mellitus affects the liver in addition to blood vessels, kidneys, retina and nerves [33, 34]. Alloxan exerts a toxic effect on pancreatic beta cells, which causes T1DM, and T2DM but this effect extends to the kidneys and livers of rat.

Diabetes mellitus is associated with increase in blood glucose level. From this present study, after the induction of alloxan on the experimental animals, there was a drastic increase in the level of glucose across the groups. This confirms the cytotoxic action of alloxan which includes damage and death of pancreatic islets cells in the animals. This study shows that at day

1, there was a significant ($p<0.05$) increase in the glucose level of negative control (364.10±0.05), positive control (390.10±0.07) and across all extract-treated groups when compared to normal control (62.00±1.22). This correlates with the findings of Ankur and Shahjad [35] that alloxan induces diabetes by destroying the insulin-producing pancreatic beta cells. However, a time-dependent significant ($p<0.05$) decrease of glucose level till the end of the experiment (28 days), across all the extract treated groups; Groups 4 (95.30±0.08), 5 (132.30±0.09) and 6 (132.30±0.06) was observed when compared to the negative control (328.00±0.03).

The result of this experiment is in line with the findings of Cazarolli *et al.*, [36] that MEACis used as the generic medicine to balance glucose levels. This denotes that the MEAC attenuates glucose level by down-regulation of glucose level.

The elevation in the serum markers namely AST, ALT and ALP is one of the causes of hepatotoxicity of alloxan [37]. Alloxan exerts a toxic effect on pancreatic beta cells, which causes Type 1 diabetes mellitus (T1DM), but this effect extends to the liver of several species. The hepatic cells are involved in a variety of metabolic activities. They consist of higher concentration of AST and ALT in the cytoplasm and AST in particular exist in mitochondria [38]. The significant reductions of these marker enzymes by MEAC are corroborated by the works of Nordby *et al.* [39], Chua *et al.*[16], Koyama [40] and Gunasekara *et al*. [17]. These effects could be as a result of the inherence of vital phytochemicals in the plants like saponins, alkaloids, and flavonoids, as outlined by Nettem *et al*. [23] and Thomas *et al.* [24]. This study has, therefore, shown that MEAC could protect the liver against alloxan-induced hepatotoxicity. The hepatoprotective effect of MEAC was evident due to the recovery of liver damage by causing accelerated regression of damage induced by alloxan.

Total protein test measures the total amount of albumin and globulin in the body. This present study showed that the levels of total protein and globulin where not altered in relation to the normal rats. The significant reduction of albumin concentration in the negative control indicates a form of hypoalbuminemia, which is associated with short term mortality and complications as shown by Lyons *et al.*, [41] and Haller [42]. However, this was attenuated by

the standard drug and MEAC (100 and 200 mg/kg) during the course of the administration possibly by improving the synthetic function of the liver.

The administration of the methanol extract of *Averrhoa carambola* leaves was found to have ameliorative effect especially on the urea concentration of alloxan-induced wistar rats. The significant reduction in urea after treatment can be attributed to the ability of the extract to reduce glucose concentration and thus increase insulin effect causing a decline in proteolysis [43]. The degenerative changes induced by alloxan monohydrate in rats' pancreas were ameliorated by MEAC and Glibenclamide in the treated groups.

CONCLUSION

The present study showed that the methanol extract of *Averrhoa carambola* leaves offered significant hypoglycemic effect and protection against hepatic damage caused by alloxan-induced diabetes. These properties of the extract may be attributed to its constituents (which are mainly polyphenolic compounds) with its antioxidant and membrane-stabilizing properties, therefore justifying their potential in traditional medicine for the management of diabetes. Further studies are required to identify the particular active phytochemicals and the precise mechanism(s) underlying the beneficial effects of these plants.

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