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Attenuative Benefits of Leaf Extract of *Annona muricata* in Alloxan-Induced Diabetic Mice

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ABSTRACT: The methanol extract of *Annona muricata* leaf was investigated for its effects on blood glucose, haematology, serum electrolyte and kidney parameters of alloxan-induced diabetic mice. Six groups of mice (5 mice per group) was used for this study. Group A served as "Normal control" and fed mice pellets and water. Diabetes was induced in Groups B, C, D, and E by intraperitoneal injection of alloxan (90 mg/kg). Group C were treated with glibenclamide (5 mg/kg body weight), group D, E, and F received 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight of extract daily respectively for 21 days via oral gavage. Group B mice were induced but not treated with any drug, thus it served as the "Negative control" group. The extract significantly ($p>0.05$) lowered the fasting blood glucose level in alloxan induced diabetic mice. The effect of diabetes on Group B 18.50 was significantly low in the final body weight (kg) of the mice compared to group C, D, E and F (51.69, 33.54, 29.23 and 33.40) which were increased. However, the group A (45.02) remained healthy throughout the study period. The extract showed significantly improved haemoglobin (Hb) values in the treatment groups compared to the diabetic untreated groups. The findings from this study suggest that methanol leaf extract of *A. muricata* demonstrated better anti-diabetic agents in mice and may be of immense benefits in the management of diabetes and its associated complications.

Keywords: Diabetes mellitus, *A. muricata*, Blood glucose level, Hyperglycemia, Mice

Introduction

Annona muricata L. is a medicinal plant that belongs to the family Annonaceae. It is grown in the rain forest regions of the South-Eastern plains and Niger-Delta basins in Nigeria. *A. muricata* is known by different local names, for example soursop (English) due to the sour and sweet taste of the fruit, graviola (Portuguese), Sirsak (Indonesia) and guanabana (Latin America). Phytochemical studies have been extensively carried out on different parts of *A. muricata* and till date, the major phytochemicals reported to be present in *Annona muricata* are alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, phytosterols, terpenoids and proteins (Edeoga *et al.*, 2005). Among the chemical constituents found in *A. muricata*, the alkaloids (reticulin, coreximine, coclarine and anomurine) (Leboeuf *et al.,* 1982) and essential oils (β-caryophyllene, δ-cadinene, epi-α-cadinol and α-cadinol) (Kossouoh, 2007). All parts of the *A. muricata* tree are used in natural medicine in the tropics including the bark, leaves, root and fruit-seeds. Ethnobotanical studies have indicated that *A. muricata* Fruit juice and infusions of leaves or branches have been used to treat fever, to increase mother's milk after childbirth (lactagogue), as an astringent for diarrhea and dysentery and possesses wound healing (Agu *et al.,* 2017), anti-arthritic, anticonvulsant (Moghadamtousi *et al.,* 2015), anti-diabetic and hypolipidemic (Adeyemi *et al.,* 2009); anti-inflammatory and anti-

nociceptive (De Sousa *et al.,* 2010, Hamid *et al.,* 2012) antioxidant (Agu and Okolie, 2017) anti-hypertensive (Nwokocha *et al.,* 2012) antiparasitic (Jaramillo *et al.,* 2000) anti-plasmodial (Ménan *et al.,* 2006) hepatoprotective (Arthur *et al.,* 2012) gastroprotective and antineoplastic effects (Agu and Okolie, 2017).The aim of this study was to investigate the blood glucose lowering effects and attenuate haematological and renal indices of methanol extracts of *Annona muricata* in an alloxan induced diabetic mice when compared with glibenclamide.

Materials and methods

Collection, identification and preparation of leaves: Fresh leaves of *Annonna muricata* used for this study were obtained from a home garden in Olokoro, in Umuahia North L.G.A., Abia State, Nigeria and identified by a taxonomist in the Department of Plant Science and Biotechnology, College of Natural Sciences (COLNAS), of Micheal Okpara University of Agriculture, Umudike, The leaves were separated from the stalk, washed and air-dried at room temperature. The leaves were constantly weighed using electronic weighing balance (JA-P Metra) until constant weights were observed, after which the dried leaves were milled to fine powder using a mechanical grinder.

Animal procurement and management: Thirty (30) balb/C albino of both sexes, weighing between 24-30 g were obtained from the Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State and housed in the animal house of the Zoology and Environmental Biology Department, Michael Okpara University of Agriculture Umudike under standard conditions of light, temperature, and humidity of 12 h lights and 12 h dark period at room temperature. The animals were given free access to standard commercial rat pellets and drinking tap water *ad libitum* and were kept for 14 days to acclimatize. Acute toxicity test of the leaf extract was also determined using modified Locke's toxicity testing method as reported by (Aroma and Enegide, 2014). The rats were divided into 6 groups of 5 rats each. 3 groups served as control groups (i.e. normal control, negative and positive control) while the other 2 groups served as the test groups and they were maintained under standard laboratory conditions throughout the period of the study.

Chemicals and solvent: All chemicals and solvent used for the study were of analytical grades and were purchased from Sigma Aldreich Co. UK, through Bristol Scientific, Ikeja Lagos, Nigeria.

Extraction of the plant leaves: Methanol extract of the plant leaves was prepared by a modified method as described by (Usunobun *et al*., 2015). One hundred (100) grams of the dry powdered plant leaves was soaked in 1000 ml of 70% ethanol solution at room temperature for 48 h (for thorough extraction). The extract was cold macerated (filtered) using muslin cloth, and then filtered through Whatman filter paper No.4. The extract was thereafter concentrated using a water-bath at 60 °C to one-tenth of its original volume. The percentage yield of the crude extract was 5.2 %. The crude extract was then stored at 4 °C in the refrigerator and subsequently used for the studies. The extract were dissolved in distilled water for use on each day of the experiment.

Animal treatment:

Animal grouping and treatment: Thirty balb/C albino mice were divided into six groups of five mice per group. Diabetes mellitus was induced in Groups B, C, D, E and F only by intraperitoneal injection of alloxan (120 mg/kg). Group A mice served as "normal control" animals and received normal rat pellets and water. Group C was treated with Glibenclamide (5 mg/kg body weight), group D, E and F received 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight of *A. muricata* leaf extract daily respectively throughout the duration of experiment of 14 days. Group B was induced but not treated with any drug, thus it served as the "negative control" group.

Induction of diabetes: The mice were fasted for 18h overnight after which diabebtes mellitus was induced with alloxan monohydrate, (120 mg/kg body weight) dissolved in 10 ml of distilled water intraperitoneally (i.p) (Etuk, 2010). Hyperglycemia was confirmed when elevated blood glucose levels were equal or greater than 200mg/dl after 48h of injection. (Trupti and Dayamand, 2014) Prior to induction of diabetes mellitus (hyperglycemia), the blood glucose was determined using Accu-Chek glucometer with compatible glucose test strips for all the animals scheduled for experiment and subsequently on alternate days (every two days) after induction of diabetes mellitus.

Administration of extract: Five thousand milligrams (5000 mg) i.e. (5 g) of the crude extract was reconstituted using 25 ml of distilled water and the concentrated extract at increasing doses of 200 mg/kg (b.wt) and 500 mg/kg (b.wt) based on body weights of the individual rats were administered to the rats through oral gavage. The administration of the extract was carried out for 14 days. Body weights of the animals were measured every two (2) days, after which the animals were sacrificed, and blood samples collected for biochemical analyses

Collection of blood sample: Blood samples were obtained by repeated lancet puncture of the tail tip veins. Fasted alloxan administered rats with blood glucose concentration greater than or equal to 200 mg/dl were considered to be diabetic and included in the study using Accu-Chek glucometer with compatible glucose test strips for all the animals scheduled for experiment.

Statistical analysis: All the data were expressed as mean ± standard deviation (SD) and statistical significance was evaluated by using t-test assuming equal variances, One way analysis of variance (ANOVA) in Microsoft Excel statistical package 2007 version. The level of significance was considered as ($p<0.05$).

Results

Effect of the leaf extract on body weight: Table. 1 Shows the effect of the methanol leaf extract of *Annona muricata* on the initial general body weight. There was no significant difference ($P>0.05$) in body weight when the glibenclamide and extract treated groups were compared with the normoglycemic group, and no significant difference ($P>0.05$) between the extract treated groups. Further shows the effect of the methanol leaf extract of *Annona muricata* on the final average body weights of the various groups of mice at the end of administration. The result did not show any significant ($P>0.05$) increase in the body weight of the extract treated groups and control groups, However, there was a significant increase ($P>0.05$) in body weight of the standard drug treated group.

Table 1: Effect of methanol extract of *A. muricata* leaves on body weight of alloxan-induced diabetic mice.

Group	Treatment	Initial BW(g)	Final BW (g)	BW Difference (g)
A	Control	22.48 ± 1.31^{a}	45.02 ± 1.48^{b}	22.54 ± 1.04^{b}
B	Diabetic Untreated	23.06 ± 0.96^{a}	18.50 ± 1.63^{d}	-4.56 ± 1.19^{d}
C	Standard Drug (Daonil)	22.04 ± 0.41^{a}	54.69 ± 1.34^{a}	32.65 ± 1.42^{a}
D	250 mg *A. muricata*	25.22 ± 0.88^{a}	33.54 ± 1.33^{c}	8.32 ± 2.16^{c}
E	500 mg *A. muricata*	24.06 ± 0.83^{a}	29.29 ± 1.65^{c}	5.23 ± 1.99^{c}
F	1000 mg *A. muricata*	24.06 ± 1.20^{a}	33.40 ± 2.28^{c}	9.34 ± 2.78^{c}

Average Body weights for the various groups of mice measured every two days (data presented as mean ± SD). Values with same letters in the same column are not significantly different at $P<0.05$.

Effect of methanol leaf extract of annona muricata on hyperglycemic mice: Table. 2 shows the effect of the methanolic leaf extract of *Annona muricata* on the glucose level of experimental mice. The result showed that the glucose levels were exceedingly high in Group B rats when compared with rats in other groups.

Table 2: Effect of methanol extract of *A. muricata* leaves on hyperglycemic rats on alloxan-induced diabetic mice.

Group	Treatment	Initial FBG (mg/dl)	Final FBG (mg/dl)
A	Control	60.20 ± 2.76^{b}	82.80 ± 3.25^{e}
B	Diabetic Untreated	53.00 ± 1.14^{d}	245.20 ± 1.50^{a}
C	Standard Drug (Daonil)	60.00 ± 1.30^{bc}	95.00 ± 0.71^{d}
D	250 mg *A. muricata*	55.00 ± 1.30^{cd}	118.80 ± 0.86^{c}
E	500 mg *A. muricata*	65.40 ± 1.03^{a}	122.00 ± 2.55^{c}
F	1000 mg *A. muricata*	59.00 ± 1.61^{bc}	130.00 ± 1.61^{b}

Table 2 shows the effect of the methanol leaf extract of *Annona muricata* on the glucose level of experimental rats. The result showed a significant ($P<0.05$) decrease in the glucose levels of the glibenclamide treated group and *A. muricata* extract treated groups. There is no significant ($P>0.05$) difference in glucose levels between the groups treated with the extract.

From the statistical analysis of the result of glucose levels of the mice in each group, it was observed that when the Normoglycemic group was compared with the Glibenclamide-treated group, 250 mg/kg treated (induced) group, there was a highly significant difference ($p<0.05$) in the decrease of glucose level, but there was no significant difference ($p>0.05$) between the Normoglycemic group and the 500 mg/kg (induced), 400 mg/kg (not induced)

group. When the diabetic (untreated) group was compared with Glibenclamide-treated group, 250 mg/kg (induced), 500 mg/kg (induced) and 1000 mg/kg (induced) group, there was a significant difference ($p<0.05$) in glucose levels as the glucose levels reduced progressively especially with increase in the concentration of the extract. There was no significant difference ($p>0.05$) in glucose level when the Glibenclamide-treated group was compared with the 250 mg/kg (induced) and 500 mg/kg (induced) group. But there was a significant difference ($p<0.05$) between the Glibenclamide group and the 10000 mg/kg (induced) group. There was no significant difference ($p>0.05$) in glucose levels between the 3 extract-treated groups.

Effect of methanol extract of A. muricata leaves on haematological parameters in alloxan-induced diabetic mice: The results of the effect of methanol leaf extract of *Annona muricata* on haematological indices in alloxan-induced diabetic mice is shown in Table 3. There was significant ($P>0.05$) decrease in the haemoglobin (HB), packed cell volume (PCV) and red blood cells (RBC) values of diabetic untreated mice when compared with control group. However, treatment with 250mg, 500mg, 1000mg and standard drug significantly increased HB, PCV and RBC levels higher than the diabetic untreated mice group. The white blood cells (WBC) value was significantly ($P>0.05$) increased in diabetic untreated mice when compared with control group. Treatment with *A. muricata* and standard drug significantly ($P>0.05$) decreased this parameter when compared to diabetic untreated mice group. MCV and MCH values of diabetic untreated mice was significantly higher than control group, on the other hand MCHC value of diabetic untreated mice was significantly lower than that of control group. Treatment with *A. muricata* and standard drug showed significant ($P>0.05$) and dose related reduction in the MCH and MCHC values.

Table 3: Effect of methanol extract of *A.muricata* leaves on hematological parameters of alloxan-induceddiabetic mice

Group	Treatment	HB (g/dl)	PCV (%)	RBC (10^6/µL)	WBC (10^3/µL)	MCV (fL)	MCH (Pg)	MCHC(g/dL)
A	Control	14.20 ± 0.12^{a}	36.50 ± 0.50^{d}	7.04 ± 0.01^{b}	5.10 ± 0.06^{f}	51.84 ± 0.63^{e}	20.17 ± 0.06^{b}	38.91 ± 0.35^{a}
B	Diabetic Untreated	8.10 ± 0.06^{f}	29.00 ± 0.33^{f}	2.20 ± 0.07^{d}	10.05 ± 0.02^{a}	131.98 ± 2.64^{a}	36.88 ± 1.01^{a}	27.94 ± 0.20^{c}
C	Standard Drug (Daonil)	10.06 ± 0.03^{c}	30.61 ± 0.58^{e}	5.36 ± 0.12^{c}	7.08 ± 0.02^{b}	57.17 ± 1.33^{d}	18.79 ± 0.40^{c}	32.86 ± 0.21^{b}
D	250mg *A. muricata*	10.30 ± 0.17^{b}	46.50 ± 0.87^{a}	7.34 ± 0.12^{a}	6.40 ± 0.13^{c}	63.36 ± 0.31^{b}	14.04 ± 0.09^{d}	22.15 ± 0.03^{d}
E	500mg *A. muricata*	9.10 ± 0.03^{e}	41.23 ± 0.38^{c}	7.02 ± 0.01^{b}	5.88 ± 0.04^{e}	58.74 ± 0.55^{cd}	12.96 ± 0.04^{d}	22.07 ± 0.22^{d}
F	1000mg *A. muricata*	9.40 ± 0.13^{d}	44.50 ± 0.83^{b}	7.26 ± 0.09^{ab}	6.15 ± 0.05^{d}	61.28 ± 0.42^{bc}	12.95 ± 0.03^{d}	21.13 ± 0.10^{e}

Values are expressed as mean ± SEM of 5 mice. Values with same letters in the same column are not significantly different $P<0.05$.

Effect of methanol extract of A. muricata leaves on liver parameters in alloxan-induced diabetic mice: Table 4 shows the effect of *A. muricata* on liver parameters of alloxan induced diabetic mice. There was significantly ($P>0.05$) elevated levels of ALT, AST and ALP found in diabetic untreated group of mice (92.17, 59.85, 25.49 U/L) when compared with control group (65.37, 41.52, 16.12 U/L). ALT, AST and ALP levels of diabetic mice treated with Standard drug (Daonil) (51.69, 22.93, 10.53 U/L) was significantly the lowest when compared with control (65.37, 41.52, 16.12 U/L). There was also significant ($P>0.05$) reduction in the level of ALT, AST and ALP in diabetic mice treated with 250mg (65.11, 33.59, 16.21 U/L), 500mg (74.12, 35.62, 15.91 U/L) and 1000mg (65.00, 34.44, 18.92 U/L) of *A. muricata* when compared with control (65.37, 41.52, 16.12 U/L) and diabetic untreated group (92.17, 59.85, 25.49 U/L). On the other hand, there was no significant difference ($P<0.05$) in the levels of total protein and bilirubin in all the groups of mice. However, there was decreased content of total protein in diabetic untreated group (4.62 g/dL), which improved in the groups of diabetic mice treated with 250mg (6.37 g/dL) and 500mg (6.44 g/dL) of *A. muricata*.

Table 4: Effect of methanol extract of *A. muricata* leaves on liver parameters of alloxan-induced diabetic mice.

Group	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	T. Protein (g/dL)	Bilirubin (mg/dl)
A	Control	65.37±2.69^{c}	41.52±4.46^{b}	16.12±0.34^{b}	6.53±0.27^{a}	0.07±0.01^{a}
B	Diabetic Untreated	92.17±2.08^{a}	59.85±0.94^{a}	25.49±0.80^{a}	4.62±0.31^{a}	0.17±0.03^{a}
C	Standard Drug (Daonil)	51.69±5.13^{d}	22.93±2.62^{c}	10.53±0.57^{c}	5.81±0.67^{a}	0.36±0.27^{a}
D	250mg *A. muricata*	65.11±2.76^{c}	33.59±3.51^{b}	16.21±1.05^{b}	6.37±0.32^{a}	0.06±0.03^{a}
E	500mg *A. muricata*	74.12±3.22^{b}	35.62±2.23^{b}	15.91±2.15^{b}	6.44±0.32^{a}	0.04±0.01^{a}
F	1000mg *A. muricata*	65.00±1.81^{c}	34.44±2.04^{b}	18.92±1.14^{b}	5.77±0.63^{a}	0.08±0.05^{a}

Values are expressed as mean ± SEM of 5 mice. Values with same letters in the same column are not significantly different P<0.05.

Effect of methanol extract of A. muricata leaves on kidney parameters in alloxan-induced diabetic mice: Table 4 shows the effect of *A. muricata* on creatinine and urea concentration of diabetic mice. The level of serum creatinine was significantly (P>0.05) increased in diabetic untreated mice (0.91±0.01 mg/dL) when compared with the control group (0.74±0.03 mg/dL). The diabetic mice that received 1000mg of *A. muricata* (0.68±0.02 mg/dL) significantly (P<0.05) reduced creatinine levels more than 250mg (0.76±0.02 mg/dL), 500mg (0.72±0.01 mg/dL) of *A. muricata* and standard drug (0.73±0.01 mg/dL). On the other hand, the concentration of urea in diabetic untreated mice (40.29±0.04 mg/dL) was significantly (P>0.05) higher when compared with control group (31.07±0.06 mg/dL). There was significant (P>0.05) reduction in urea levels of diabetic mice treated with standard drug (33.56±1.45 mg/dL), 250mg (34.71±0.26 mg/dL), 500mg (29.95±0.04 mg/dL) and 1000mg (31.19±0.10 mg/dL) of *A. muricata* when compared with the diabetic untreated group (40.29±0.04 mg/dL). Treatment with 500mg (29.95±0.04 mg/d) and 100omg (31.19±0.10 mg/dL) of *A. muricata* had the highest effect on reducing urea levels, as they did not significantly differ from control group (31.07±0.06 mg/dL).

Table 5: Effect of methanolic extract of *A. muricata* leaves on kidney parameters of alloxan-induced diabetic mice.

Group	Treatment	Creatinine (mg/dl)	Urea(mg/dl)
A	Control	0.74±0.03^{b}	31.07±0.06^{c}
B	Diabetic Untreated	0.91±0.01^{a}	40.29±0.04^{a}
C	Standard Drug (Daonil)	0.73±0.01^{bc}	33.56±1.45^{b}
D	250mg *A. muricata*	0.76±0.02^{b}	34.71±0.26^{b}
E	500mg *A. muricata*	0.72±0.01^{bc}	29.95±0.04^{c}
F	1000mg *A. muricata*	0.68±0.02^{d}	31.19±0.10^{c}

Effect of methanolic extract of A. muricata leaves on serum electrolytes of alloxan-induced diabetic mice: Table 4 shows the effect of *A. muricata* on serum electrolytes (Na^+, k^+, Cl^+ and HCO_3) of diabetic mice. Na^+ concentration of diabetic untreated mice (133.00±1.16 mEq/L) was non-significantly (P>0.05) higher when compared with control group (125.00±0.58 mEq/L) and non- significantly (P>0.05) lower when compared with diabetic mice treated with standard drug (135.00±1.16 mEq/L). Treatment with 1000mg of *A. muricata* significantly (P>0.05) reduced Na^+ levels (115.50±0.87 mEq/L) compared with other treatments- 250mg (127.50±0.87 mEq/L), 500mg (123.00±0.58 mEq/L) and standard drug (135.00±1.16 mEq/L). Diabetic untreated mice also had significantly (P>0.05) higher concentration of K^+ ion compared with control group and standard drug treated group. Treatment with 1000mg of *A. muricata* significantly (P<0.05) increased K^+ levels (16.98±0.02 mEq/L) compared with other treatments- 250mg (15.31±0.17 mEq/L), 500mg (14.89±0.02 mEq/L). There was a significant (P>0.05) decrease in serum Cl^- level of diabetic untreated mice compared to control group and diabetic mice treated with standard drug. 250mg (77.56±1.18 mEq/L), 500mg (86.66±0.43 mEq/L) and 1000mg (84.05±0.03 mEq/L) of *A. muricata* treatment on diabetic mice significantly reduce serum Cl^- when compared with control group (102.35±0.66 mEq/L). However, 250mg had the highest activity compared to all other treatments. HCO_3 levels of diabetic mice treated with standard drug (12.77±0.44 mEq/L) was significantly (P>0.05) lower when compared with control (18.65±0.24 mEq/L) and diabetic untreated (17.76±0.15 mEq/L) group. Treatment with 250mg (17.31±0.11 mEq/L) of *A. muricata* on diabetic mice significantly (P>0.05) reduced HCO_3 levels while 500mg (19.00±0.03 mEq/L) and 1000mg (18.32±0.14 mEq/L) significantly (P>0.05) increased it when compared with control group (18.65±0.24 mEq/L).

Table 6: Effect of methanolic extract of *A. muricata* leaves on serum electrolytes of alloxan-induced diabetic mice.

Group	Treatment	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	HCO_3^- (Mmol/L)
A	Control	125.00±0.58^{ab}	7.97±0.03^{d}	102.35±0.66^{a}	18.65±0.24^{b}
B	Diabetic Untreated	133.00±1.16^{a}	15.15±0.37^{b}	71.25±0.66^{f}	17.76±0.15^{bc}
C	Standard Drug (Daonil)	135.00±1.16^{a}	12.85±0.10^{c}	80.90±0.52^{d}	12.77±0.44^{d}
D	250mg *A. muricata*	127.50±0.87^{b}	15.31±0.17^{b}	77.56±1.18^{e}	17.31±0.11^{c}
E	500mg *A. muricata*	123.00±0.58^{b}	14.89±0.02^{b}	86.66±0.43^{b}	19.00±0.03^{a}
F	1000mg *A. muricata*	115.50±0.87^{c}	16.98±0.02^{a}	84.05±0.03^{c}	18.32±0.14^{ab}

Values are expressed as mean ± SEM of 5 mice. Values with same letters in the same column are not significantly different at $P<0.05$.

Discussion

From the animal study, it could be observed that the methanol leaf extract of *A. muricata* caused a reduction of the blood glucose level of the extract treated experimental rats and this indicates that the extract is a very potent blood glucose-lowering agent largely comparative to the standard drug (Glibenclamide) used in the study, at a higher dose based on the body weight. These results agrees with the study carried out by (Adeyemi *et al.,* 2009; Arthur *et al*., 2011 and Opara *et al.,* 2021). It could be observed from the study that methanol leaf extract of *Annona muricata* lowers the blood glucose levels in alloxan induced diabetic rats which is in agreement with the work of Adewole and Ojewole (2009), than standard drug (glibenclamide) used for the study, this could be as a result of the presence of antidiabetic phytochemicals such as terpenoids, phenols, alkaloids, flavonoids in the leaf extract as previously reported by Gaikwad *et al.* (2014).

From the animal study conducted, it was observed that there was no significant weight gain in the animals throughout the duration of the study, this is consistent with the study carried out by (Opara *et al.,* 2021 and Usunobum *et al*., 2015). That the leaf extract of *A. muricata* has increased fiber content, and the no significant difference in weight gain obtained from the statistical analysis justifies the claims of Arthur *et al.* (2011) that the ethanolic crude extract at higher doses such as 200 mg/kg and 400 mg/kg per day may be metabolized into toxic end product which interferes with gastric function and decreased food conversion efficiency, thus, this could lead to loss of appetite and decreased body weight. It is therefore suggested that *Annona muricata* leaf extract possess hypoglycemic properties and is useful in the management of diseases such as diabetes mellitus and other diseases resulting from diabetic complications.

The effect of *Annona muricata* on some haematologic parameters of mice is as shown in Table 3. In this study, there was significant ($P>0.05$) decrease in the haemoglobin (HB), packed cell volume (PCV) and red blood cells (RBC) values of diabetic untreated mice while the reverse was observed with the white blood cells (WBC) values when compared with control group. However, treatment with 250 mg, 500 mg, 1000 mg of *A. muricata* and standard drug significantly increased HB, PCV and RBC levels and decreased significantly WBC values when compared with the diabetic untreated mice group. The observed non-significant increase in WBC count and lymphocytes could emphasize the beneficial effect of *Annona muricata* in improving the immunity and general well-being of the animals. Impaired production of erythropoietin from kidney in diabetic condition that alters the process of erythropoiesis could have resulted in decrease RBC count of diabetic mice (Thomas, 2008). The decrease in RBC count could also be the reflection of low HB content in alloxan-induced diabetic mice (Muhammad and Shamim, 2013). On the other hand, increased WBC count observed in diabetic untreated groups may be due to the pancreatic inflammation or necrosis associated with alloxan-induced diabetes (Tanko *et al.,* 2011). Treatment with *A. muricata* was able to reverse these changes, thus indicating that *A. muricata* can improve immunity function and decrease inflammation. Also, the observed non-significant difference in haemoglobin concentration in mice could be used to justify the fact that *Annona muricata* at all doses does not induce anaemia, making it safe. Also contrary to documented use of *A. muricata* in floristic studies in Ghana as a tonic (Mshana *et al.,* 2000), such was not observed in the current study. *Annona muricata* did not confer significant increase in the production of red blood cell *Annona muricata* conferred significant improvement in RBC count in test groups which reflected on the other parameters of complete blood profile like PCV, MCV, MCH and MCHC in test groups while reduced levels of WBC indicates the decrease in pancreatic inflammation or necrosis.

The liver releases alanine aminotransferase (ALT) and an elevation in plasma concentration is an indicator of liver damage. The liver and heart release AST and ALT, and an elevation in the levels of these enzymes are indicators of

liver and heart damage (Crook, 2006). The non-significant changes in ALT and AST in the treated mice at all doses (Table 4) indicates that the extract had no deleterious effect on liver function. ALP which has both hepatic and bone sources, showed a significant decrease ($p<0.05$) in mice administered 1000 mg/kg *Annona muricata*. The general lack of significant changes in the aminotransferases and ALP together with normal liver weight is an indication that *Annona muricata* is safe, offers no deleterious effect on the liver and is able to protect the liver tissues there by improves the liver function. This finding is consistent with the study carried out by Arthur *et al.* (2011), There was no significant difference ($P<0.05$) in the levels of total protein and bilirubin in all the groups of mice. However, there was decreased level of total protein in diabetic untreated group, which improved in the groups of diabetic mice treated with standard drug, 250mg and 500mg of *A. muricata*. Improvement in protein profile observed in *A. muricata* extract and standard drug treated diabetic mice may be due to marked change in amino acid metabolism (Mallikarjuna *et al.*, 2002). *A. muricata* by improving the secretion of insulin exert the protein sparing effect and reverse the altered protein profile that was very similar to that of the standard drug.

The level of serum creatinine and urea was significantly ($P>0.05$) increased in diabetic untreated mice when compared with the control group. Marked increase in serum urea and creatinine noticed in this study in alloxan-induced mice may be an indication of functional renal damage, (Usunobun and Okolie, 2016), especially by renal infiltration mechanism (Arthur *et al.*, 2011). However, *A. muricata* treatment given to alloxan-induced mice significantly lowered the serum urea and creatinine levels, thus enhancing renal function.

Serum electrolyte levels in alloxan-induced mice was altered when compared with the normal mice. Serum sodium levels were significantly ($P>0.05$) decreased in alloxan-induced diabetic mice, whereas, serum potassium levels were increased significantly as compared to control group. Some researches has reported that there is an inverse relationship exist between sodium and potassium levels. This disorder maybe based on the movement of electrolytes between intra and extra cellular spaces dependent on insulin action as well as hyperosmolarity. Treatment with standard drug and *A.muricata* extract significantly reversed these changes (Syed and Tabassum, 2007).

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