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Possible Potency of *Annona muricata* L. Methanol Leaf Extract in Ameliorating Pancreatic β Cell Function in Alloxan-induced Diabetic Mice

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ABSTRACT: The methanol extract of *Annona muricata* leaf was investigated for its effects on β -cell dysfunction, and atherogenenic dyslipidaemia in alloxan-induced diabetic mice. Thirty (30) mice of both sexes, aged between 8-10 weeks were randomly divided into normal, diabetic, diabetic + glibenclamide(5mg/kg), diabetic+250 mg/kg *A. muricata* Extract, diabetic+500 mg/kg *A. muricata* Extract, diabetic+1000 mg/kg *A. muricata* Extract, groups (n=6/group). Alloxan was administered intraperitoneally while extracts and glibenclamide were given via oral route for 21 days. The results revealed that, 250 mg/kg *A. muricata* extract elicited significant reductions in hyperglycemia, ameliorated insulinaemia as well as β -cell function and impaired weight loss in alloxan-induced diabetic mice. The extract also attenuated ($p<0.05$) atherogenic dyslipidemia and elevation in malondialdehyde and upregulated antioxidant enzymes. The effect of the extract on the test groups administered 250 mg/kg body weight (bw), 500 mg/kg bw, and 1000 mg/kg bw compared favourably with glibenclamide. The administration of methanolic extract of *A. muricata* leaves has significant ameliorative effect on alloxan-induced hypoglycemia, atherogenic dyslipidaemia and pancreatic β cell dysfunction. Our finding suggest that these positive modulatory effects are achieved via reduction in lipid peroxidation. The extract may be of immense benefits in the management of diabetes and its associated complications. Improved liver functions as well as its anti-oxidant status are beneficial in the management of chronic diseases.

Keywords: Diabetes mellitus, *A. muricata*, β-cell dysfunction, Atherogenenic dyslipidaemia

Introduction

Diabetes mellitus is a chronic condition, characterized by hyperglycemia, occurring due to impaired insulin secretions. It is the most common metabolic disease worldwide (Walker and Whittlesea, 2012). Most Antidiabetic drugs available today are synthetic in origin and thus cost more and show toxicity (Diptanu *et al.,* 2016). Various studies have revealed variety of antidiabetic plants useful in the management and treatment of diabetes mellitus in various indigenous systems of medicine (Deb *et al.,* 2013). *Annona muricata* is a member of the Annonaceae family and is a fruit tree with a long history of traditional use. *Annona muricata* has shown to have both medicinal and industrial value (Mishra *et al.,* 2013; Soheil *et al.,* 2015). In ethno-medicine, *A.muricata* leaves used by local traditional healers in treatment of various ailments such as gastric discomfort, stomach ulcer, diarrhea, dysentery and skin infections (De Sousa *et al.,* 2010; Soheil *et al.,* 2015). Phytochemical analysis of *A.muricata* showed the presence of flavonoid, anthraquinone, alkaloids, saponins, steroids, terpenoids, cardiac glycoside, anthocyanin, tannins and carotenoid in both the aqueous and ethanolic extracts (Nawwar *et al.,* 2012; Yang *et al.,* 2015). Other studies have revealed that *A.muricata* extracts has antioxidant potential. Consequently, it has become imperative to investigate the plant for the management of

diabetes. Presence of flavonoids and tannins in the extracts suggests they possess antidiabetic activity (Nawwar *et al.,* 2012; Jiménez *et al.,* 2014).

Materials and Methods

Sample collection and preparation of extract: Fresh leaves of *A. muricata* used for this study were obtained from Olokoro village, Umuahia North L.G.A., Abia State in January 2017. A plant taxonomist (Dr. Mulikat A. Jimoh) in the Department of Plant Science and Biotechnology, College of Natural Sciences (COLNAS), Michael Okpara University of Agriculture Umudike, identified and authenticated the leaves as *A. muricata.* The fresh leaves of *A. muricata* were washed with tap water; room dried for one month, and macerated. The macerated samples (615 g) was soaked in 98% methanol and allowed to stand for 5days in a bell jar, shaken intermittently and filtered. The filtrate was evaporated to dryness at reduced temperature of 40°C in a water bath to yield a semisolid mass, which was stored in a refrigerator until required for use.

Experimental animals: Thirty (30) male mice, weighing about 24 – 30 g were purchased from the animal house, Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. They had unrestricted access to standard feed and water. The animals were maintained under standard environmental conditions of temperature, relative humidity and dark/light cycle, in accordance with the guidelines of National Institute of Health Guide for the Care and Use of Laboratory Animals and the Institutional Ethical Review Board of Michael Okpara University of Agriculture Umudike. Body weight, food consumption and water intake were monitored throughout the period of Administration. The animals were acclimatized to the condition of their new environmental prior to treatment for three weeks.

Induction of diabetes: The mice were fasted overnight and then injected, intra-peritoneally, with a single dose 0.24 g of alloxan dissolved in 10 mL of distilled water was administered to each of the mice in groups A, B, C, D, E and F based on the body weight of each mice and on a dosage of 100 mg/kg. Group A served as the control. The administration of alloxan was done intraperitoneally using diabetic syringes. Stable hyperglycemia was confirmed on the 5th day using glucometer. Mice with fasting blood glucose level greater than 180mg/dl were considered hyperglycemic and used for the study.

Animal grouping and treatment: After 2 weeks of acclimatization, animals were randomly assigned to six groups (n=6/group). Group A (normal control) and B (diabetic control):received distilled water (vehicle) daily; Group C: (diabetic + glibenclamide): received 5.0 mg/kg body weight of standard drug (glibenclamide) ;Group D: (diabetic + 250 mg/kg *A. muricata* extract): received 250mg/kg body weight of methanol *A. muricata* extract; Group E: (diabetic + 500 mg/kg *A. muricata* extract): received 500mg/kg body weight of methanol *A. muricata* extract; while Group F: (diabetic + 1000 mg/kg *A. muricata* extract): received 1000 mg/kg body weight of the methanol *A. muricata* extract. All treatments were through oral administration and lasted for 21 days.

Blood collection: At the end of experiment, four (4) mice, from each group, were weighed and sacrificed by cervical dislocation while under mild anesthesia and blood was collected by cardiac puncture into plain bottle. The blood was left undisturbed for 5 min and then centrifuged at 3500rpm for 10min and serum was stored frozen until needed for biochemical assays.

Biochemical assays: Insulin was determined using ELISA kit from Ray Biotech, Inc. (Georgia USA), while malon-dialdehyde (MDA), a maker of lipid peroxidation was measured by standardized method using kit obtained from Oxford Biomedical Research Ltd (Rochester Hills, MI). Estimation of total cholesterol (TC-) and Triglyceride (TG) was done by standardized enzymatic colorimetric methods using assay kits obtained from Fortress Diagnostic Ltd. (Antrium, United Kingdom). High-density lipoprotein-cholesterol (HDL-C) was measured by enzymatic clearance assay (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) while low-density lipoprotein-cholesterol (LDL-C) was estimated using modified Friedewald's formular. TC/HDL-C and TG/HDL-C ratios were estimated as markers of atherogenic lipids indices

Gross necropsy and general observation: All the animals in the study were subjected to a determined gross necropsy, which included careful examination of the external surface, examination of the external surface of the body, all orifices and cranial, thoracic and abdominal cavities. The behaviour depression, salivation, diarrhea and muscular weakness were observed throughout the experimental period.

Fasting blood glucose and pancreatic β cells: Weekly fasting blood glucose was determined by glucose oxidase method of Trinder (1969) using ACCU-CHEK, Active Blood glucose monitoring system (Roche Diagnostics GmbH Sandhofer Strasse 1158305 Mannham, Germany). The pancreatic β cells function was estimated using HOMA- B formular

Statistical analysis: Data were analyzed and presented as mean ± SEM. Data were subjected to statistical analysis using one-way analysis of variance (ANOVA). Values were considered statistically significant at P<0.05.

Results

Table 1a shows the effect of methanolic extract of *A. muricata* on fasting blood glucose concentration of diabetic mice. Alloxan treatment resulted in a significant (P>0.05) increase in blood glucose level in group B animals from (53.00±1.14 - 245.20±1.50 mg/dl). These values were considerably higher than that of the control group (82.80±3.25 mg/dl). Oral administration of methanolic extract of *A. muricata* at the dose 250 mg (118.80±0.86 mg/dl), 500 mg (122.00±2.55 mg/dl), and 1000 mg (130.00±1.61 mg/dl) significantly (P>0.05) decreased blood glucose level when compared with diabetic untreated mice group (245.20±1.50 mg/dl).

Table 1a: Effect of methanolic extract of *A. muricata* leaves on fasting blood glucose (FBG) in 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	0.5 mg/kg Standard Drug (Daonil)	60.00±1.30^{bc}	95.00±0.71^{d}
D	250mg/kg *A. muricata*	55.00±1.30^{cd}	118.80±0.86^{c}
E	500mg/kg *A. muricata*	65.40±1.03^{a}	122.00±2.55^{c}
F	1000mg/kg *A. muricata*	59.00±1.61^{bc}	130.00±1.61^{b}

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

The effect of methanolic extract of *A. muricata* on insulinemia of diabetic mice is presented in Table 1b. Fasting glycemia was kept above 200 mg/dl in both diabetic control, 500 mg/kg and 1000 mg/kg *Annona muricata* group, while glibenclamide and 250 mg/kg treatments lowered fasting glycemia. Meanwhile alloxan reduced circulating insulin, while administration of glibbenclamide and 250mg/kg *Annona muricata* elevated blood insulin level

Table 1b: Effect of methanolic extract of *A. muricata* leaves on insulinemia in alloxan-induced diabetic mice

Group	Treatment	Initial INS (µUI/mL)	Final INS (µUI/mL)
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	0.5mg/kg Standard Drug (Daonil)	60.00±1.30^{bc}	95.00±0.71^{d}
D	250mg/kg *A. muricata*	55.00±1.30^{cd}	118.80±0.86^{c}
E	500mg/kg *A. muricata*	65.40±1.03^{a}	122.00±2.55^{c}
F	1000mg/kg *A. muricata*	.00±1.61^{bc}	130.00±1.61^{b}

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

Table 2 shows the effect of methanolic leaves extract *A. muricata* on body weight of alloxan-induced diabetic mice. No significant (P>0.05) changes were observed in the initial body weight of mice used for this study. However, there was significant decrease in the final body weight and body weight difference of diabetic untreated mice when compared with control group. On the other hand, there was significant (P>0.05) increase in the final body weight and body weight difference of mice treated with standard drug when compared with control group. The diabetic groups of mice treated with 250, 500 and 100 mg of *A. muricata* did not show any significant difference when comparing amongst themselves but a significant decrease was observed when compared with control group.

Table 2: Effect of methanolic extract of *A. muricata* leaves on body weight in 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	0.5mg/kg Standard Drug (Daonil)	22.04±0.41[a]	54.69±1.34[a]	32.65±1.42[a]
D	250mg/kg *A. muricata*	25.22±0.88[a]	33.54±1.33[c]	8.32±2.16[c]
E	500mg/kg *A. muricata*	24.06±0.83[a]	29.29±1.65[c]	5.23±1.99[c]
F	1000mg/kg *A. muricata*	24.06±1.20[a]	33.40±2.28[c]	9.34±2.78[c]

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

The effect of *A. muricata* extract and glibenclamide on serum lipid profile in alloxan-induced diabetic mice is shown in Table 3. There was significant (P<0.05) increase in the serum triglycerides (TAG), total cholesterol (CHOL), low density lipoprotein (LDL) levels and very low density lipoprotein (VLDL) levels, and a decrease in the HDL cholesterol levels was observed in diabetic untreated mice when compared to the normal mice group (control). Significant reductions of TAG, CHOL, LDL and VLDL (45.10, 60.63, 18.94 and 9.02 mg/dl), were observed in drug treated group of mice compared to diabetic untreated group which had (85.90, 97.96, 59.93 and 17.18 mg/dl). Elevated serum TAG level decreased significantly in 250mg (48.77 mg/dl), 500mg (44.25 mg/dl) and 1000mg (46.24 mg/dl) of *A. muricata* treated group when compared to diabetic untreated mice (85.90 mg/dl) and was not significantly different from control group (47.11 mg/dl). The diabetic mice treated with 500mg and 1000mg of *A. muricata* showed highest activity observed by the significant (*P*>0.05) decrease in serum CHOL level (44.57 and 41.36 mg/dl) when compared to 250mg of *A. muricata* (60.59mg/dl) and drug treated group (60.63mg/dl). Similarly, 500mg (44.57 mg/dl) and 1000mg (41.36 mg/dl) of *A. muricata* treated mice had significantly lower CHOL levels compared with control (57.71mg/dl) and diabetic untreated mice (97.96 mg/dl). Treatment with 250mg, 500mg and 1000mg significantly (P>0.05) increased serum HDL level (31.24, 28.63 and 27.39 mg/dl) and decreased elevated LDL level (19.60, 7.09 and 4.73 mg/dl) when compared to diabetic untreated group for HDL (20.85mg/dl), LDL (59.93mg/dl) and control for HDL (35.34mg/dl), LDL (12.95mg/dl). Similarly, 250mg, 500mg, 1000mg of *A. muricata* and drug treated mice significantly (P>0.05) reduced VLDL levels (9.75, 8.85, 9.25 and 9.02mg/dl) when compared with diabetic untreated mice (17.18 mg/dl) and was not significantly (P>0.05) different from control group (9.42 mg/dl).

Table 3: Effect of methanolic extract of *A. muricata* leaves on lipid profile of alloxan-induced diabetic mice

Group	Treatment	TAG (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
A	Control	47.11±0.55[b]	57.71±1.48[b]	35.34±0.07[a]	9.42±0.11[b]	12.95±1.39[bc]
B	Diabetic untreated	85.90±2.16[a]	97.96±1.32[a]	20.85±0.54[f]	17.18±0.43[a]	59.93±2.10[a]
C	0.5mg/kg standard drug (Daonil)	45.10±1.08[b]	60.63±5.92[b]	32.67±0.20[b]	9.02±0.22[b]	18.94±5.83[b]
D	250 mg/kg *A. muricata*	48.77±4.49[b]	60.59±6.38[b]	31.24±0.07[c]	9.75±0.90[b]	19.60±6.26[b]
E	500 mg/kg *A. muricata*	44.25±2.79[b]	44.57±2.78[c]	28.63±0.23[d]	8.85±0.56[b]	7.09±3.23[bc]
F	1000 mg/kg *A. muricata*	46.24±2.62[b]	41.36±1.87[c]	27.39±0.37[e]	9.25±0.52[b]	4.73±1.24[c]

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

Table 4 shows the effect of *A. muricata* on antioxidant parameters - superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) of diabetic mice. There was significant (P>0.05) decrease in the level of SOD (4.34±0.01 U/mg), CAT (5.49±1.37 µmol/mg) and MDA (0.34±0.07 µmol/mg) of diabetic untreated mice when compared to their control groups (19.54±0.28 U/mg, 18.71±0.26 and 2.16±0.06 µmol/mg respectively). Treatment with 250, 500 and 1000 mg 0f *A. muricata* significantly (P>0.05) increased SOD, CAT and MDA levels of the diabetic mice. However, as the concentration of *A. muricata* given to the diabetic mice increased from 250 mg – 1000 mg, the concentration of SOD significantly (P>0.05) reduced from (11.17±0.08 to 8.67±0.20 U/mg), CAT from (12.99±0.02 to 7.01±0.55 µmol/mg), while MDA from (1.00±0.01 to 0.67±0.03 µmol/mg).

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

Groups	Treatments	SOD (U/mg)	CATALASE (µmol/mg)	MDA (µmol/mg)
A	Control	19.54±0.28[a]	18.71±0.26[a]	2.16±0.06[a]
B	Diabetic Untreated	4.34±0.01[e]	5.49±1.37[d]	0.34±0.07[e]
C	0.5mg/kg Standard Drug (Daonil)	13.24±0.01[b]	13.27±0.30[b]	1.04±0.01[b]
D	250mg/kg *A. muricata*	11.17±0.08[c]	12.99±0.02[b]	1.00±0.01[b]
E	500mg/kg *A. muricata*	10.94±0.11[c]	8.22±0.05[c]	0.84±0.02[c]
F	1000mg/kg *A. muricata*	8.67±0.20[d]	7.01±0.55[c]	0.67±0.03[d]

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

Discussion

In Africa and the world at large diabetes mellitus is one of the leading causes of death. Currently, available synthetic drugs used by diabetic patients have a number of limitations, such as adverse effects and high rate of secondary failure. The use of plants, plants extract, and the active compound from plants to cure diseases is a potential step in new drugs discovery (Rupeshkumar *et al.,* 2014). Different researchers suggested and proved the efficacy of plant extract and phytochemicals in diabetes treatment (Ganesh *et al.,* 2010).

This study investigated the possible potency of *Annona muricata* methanolic leaf extract in ameliorating pancreatic β cell function and dyslipidemia in type 2 diabetic mice, using alloxan-induction. Alloxan is a glucosamine-nitrosourea derived from Streptomyces achromogenes (gram-positive bacterium), and it is used for the treatment of pancreatic beta cell carcinoma. Alloxan inducing diabetes, hyperinsulinemia, or hyperglycemia by damaging the pancreatic beta cell.

It stimulates insulin-dependent diabetes (T2D) by inducing selective necrosis of the beta-cells of pancreatic islets, thus, destroying β-cells and reducing their function. From our findings *A. muricata* methanolic extract, alloxan-induced hyperglycemia at low dose (250 mg/kg), which compared favourably with glibbenclamide.

Our result showed that alloxan treatment significantly (P>0.05) increased blood glucose level of mice thereby leading to diabetes (Table 1a). The increase in blood glucose level may be a result of the shrinking, destruction, ballooning, picnosis, and necrosis of the beta cells of the islet of Langerhans (Eliakim and Obri, 2009; Saha *et al.,* 2016). However, treatment of diabetic mice with oral administration of 250, 500 and 1000 mg/kg doses of methanolic L. extract of significantly (P>0.05) decreased blood glucose level of alloxan induced diabetic mice and improved pancreatic B cell function and thus, insulin production, better than the standard drug (glibenclamide) used. This may imply that, the leaf extract of *A. muricata* induced regeneration of pancreatic B cell and hence, enhanced its function. This result agrees with Sovia *et al.* (2017) that shows *A. muricata* to have hypoglycemic effect. Hypoglycemic effect of *Annona muricata* leaves extract could be due to the presence of flavonoids in the leaves extract. Flavonoids increase insulin secretion as well as preventing beta-cell apoptosis, and modulation of proliferation (Castell *et al.,* 2008). Flavonoids can also stimulate Ca^{2+} uptake from isolated islet cells, hence it also effective even in non-insulin dependent diabetes (Sandhar *et al.,* 2011).

There was significant decrease in the final body weight and body weight difference of diabetic untreated mice when compared with control group (Table 2). The decrease in body weight observed in diabetic untreated mice is linked to the result of degradation of proteins (muscle wasting). Structural proteins are known to contribute to the body weight. In the absence of glucose and lipid sources, proteins are the next main source of energy in body. Therefore, it is clear that the decrease in body weight in diabetic mice were mainly because of degradation of structural proteins (Ananthi *et al.,* 2003; Pries *et al.,* 2006). It was observed that the body weights of mice found in three test groups treated with 250, 500, and 1000 mg/kg of *A. muricata* improved hence the extract may also help to restore body tissue proteins.

Atherogenic indices (TC/HDL-C and TG/HDL-C ratios) have been identified as successful markers for predicting individuals at the risk of atherosclerosis, which begins early in life. Therefore, our result which showed that alloxan increased TC/HDL-C and TG/HDL-C ratios and that treatment with the leaf extract of *A. muricata* attenuated rise in the ratios are very prominent. The result suggest that, monitoring TC/HDL-C and TG/HDL-C ratios may assist in early detection of associated atherogenic dyslipidemia in diabetics and that, leaf extract of *A. muricata* could serve as a good therapeutic agent for early intervention against cardio-metabolic disorders.

Significant elevation of TG, TC, LDL and VLDL and decreased level of HDL in alloxan induced diabetic mice was observed. Treatment with methanolic extract of *A. muricata* leaves not only decreased serum TG, TC, LDL and VLDL but also increased HDL level significantly. The level of serum lipid profiles are usually high in diabetic mice and such elevation represents risk factor for coronary heart diseases (Maruthupandian *et al.,* 2010). The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Kanakasabapathi and Gopalakrishnan, 2015). In normal metabolism, insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic mice after *A. muricata* treatment maybe directly attributed to improvements in insulin levels (Maruthupandian *et al.,* 2010). Other research works has also reported that the flavonoids, alkaloids and tannins are responsible for hypoglycemic and hypolipidemic effect (Adeneye and Olagunju, 2009; Kanakasabapathi and Gopalakrishnan, 2015). Therefore, the presence of high amount of flavonoids in *A. muricata* (Vijayameena *et al.,* 2013) cause hypolipidemia which removes the LDL cholesterol from blood by increasing the LDL receptor densities in the liver and by binding to lipoprotein B (El-Tantaway *et al.,* 2009).

The levels of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were significantly ($p<0.05$) reduced in alloxan induced mice. These adverse changes were significantly ($P>0.05$) reversed in groups of mice treated with methanoilc extract of *A. muricata* leaves. Superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) play an important role as protective enzymes against free radical formation in tissues (Mohan *et al.*, 2010). This result suggests *A. muricata* leaves extract to have a protective role in decreasing lipid peroxidation and by normalizing antioxidant system.

Conclusion

This study showed that methanol extract of *Annona muricata* leaves has the potency of ameliorating pancreatic β-cell function and attenuated dyslipidemia in T2D condition by decreasing lipid peroxidation and by normalizing antioxidant system. This experiment backs the traditional usage of this plant leaves as an alternative drug source, for effective control/treatment of hypoglycemic and hypolipidemic agent and cardiovascular diseases.

References

Adeneye AA, Olagunju JA: Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *Carica papaya* Linn. in wistar albino rats. Biol Med, 1:1-10. 2009.

Adewole S, Ojewole J: Protective effects of *Annona muricata* Linn. (annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. Afr J Tradit Complement Altern Med, 6: 30–41. 2009.

Adewole SO. Caxton-Martins EA: Morphological changes and hypoglycemic effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on pancreatic β-cells of streptozotocin-treated diabetic rats. Afr J Biomed Res, 9: 173–187. 2006.

Adeyemi DO, Komolafe OA, Adewole OS, Obuotor EM, Adenowo TK: Anti-hyperglycemic activities of *Annona muricata* (Linn). Afr. J. Tradit. Complement Altern. Med., 6: 62–69. 2009.

Adeyemi DO, Komolafe OA, Adewole SO Obuotor EM: Anti hyperlipidemic activities of *Annona muricata* (Linn). Internet J Altern Med, 7: 1. 2008.

Adeyemi DO, Komolafe OA, Adewole SO, Obuotor EM, Adenowo TK: Effects of *Annona muricata* (Linn) on the morphology of pancreatic islet cells of experimentally-induced diabetic wistar rats. Internet J Altern Med, *5*: 2. 2008a.

Aggarwal N, Shishu: A review of recent investigations on medicinal herbs possessing antidiabetic properties. J Nutr Disorder Ther, 1:102. 2011.

Ahalya B, Shankar, KR, Kiranmayi G: Exploration of anti-hyperglycemic and hypolipidemic activities of ethanolic extract of *Annona muricata* bark in alloxan induced diabetic rats. Int J Pharm Sci Rev Res, 25:21–27. 2014.

Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC, Flores-Saenz JL: Study of the anti-hyperglycemic effect of plants used as antidiabetics. J Ethnopharmacol, 61: 101-110. 1998.

American Diabetes Association: Diabetes is a Silent Killer. Available at: www.diabetes.org. Accessed September 2017.

Ananthi J, Prakasam A, Pugalendi V: Antihyperglycemic activity of *Eclipta alba* leaf on alloxan induced diabetic rats. Yale J Biol Med, 76: 97-102. 2003.

Asare GA, Afriyie D, Ngala RA, Abutiate H, Doku D, Mahmood SA, Rahman H: Antiproliferative activity of aqueous leaf extract of *Annona muricata* L. On the prostate, BPH-1 cells, and some target genes. Integr Cancer Ther, 14: 65–74. 2015.

Atangwho IJ, Ebong PE, Eyong EU, Asmawi MZ, Ahmad M: Synergistic antidiabetic activity of *Vernonia amygdalina* and *Azadirachta indica*: Biochemical effects and possible mechanism. J Ethnopharmcol, 141(3):878–887. 2012.

Azmi MB, Qureshi SA: (2012). Methanolic root extract of *Rauwolfia serpentina* Benth improves the glycemic, antiatherogenic, and cardioprotective indices in alloxan-induced diabetic mice. Adv Pharmacol Sci, Article ID 376429. 11pages. 2012.

Baynes HW: Classification, pathophysiology, diagnosis and management of Diabetes mellitus. J Diabetes Metab, 6: 541. 2015.

Bidla G, Titanji V, Joko B, el-Ghazali G, Bolad A, Berzins K: Antiplasmodial activity of seven plants used in African folk medicine. Indian J Pharmacol, 36: 245–246. 2004.

Castell A, Baiges I Ard A. Bioactivity of flavonoids on cells. Compr Rev Food Sci Food Saf, 7:299–308. 2008.

Chan P, Ah R, Mh K: Anti-arthritic activities of *Annona muricata* L. leaves extract on complete Freund's adjuvant (CFA)-induced arthritis in rats. Planta Med, 76:166. 2010.

Chehade J, Mooradian A: A rational approach to drug therapy of type 2 diabetes mellitus. Drugs, 60: 95–113. 2000.

Chueh WH, Lin JY: Berberine an isoquinoline alkaloid in herbal plants, protects pancreatic islets and serum lipids in nonobese diabetic mice. J Agric Food Chem, 59:8021-7. 2011.

De Sousa OV, Vieira GDV, de Pinho JDJR, Yamamoto CH, Alves MS: Antinociceptive and anti-inflammatory activities of the ethanol extract of *Annona muricata* L. leaves in animal models. Int J Mol Sci, 11:2067–2078. 2010.

Deb L, Bhattacharjee C, Shetty SR, Dutta A: Evaluation of anti-diabetic potential of the Syzygium cumini (Linn) skeels by reverse pharmacological approaches. Bull Pharm Res Inst, 3:135-45. 2013.

Diptanu B, Shivraj TG, Narayanaswamy LG: Insignificant effect of ethanol extract of *Dipterocarpus turbinatus* (Dipterocarpaceae) bark on selected parameter in alloxan-induced diabetic rats. J Pharm Negat Results, 7(1): 29-32. 2016.

Eliakim-Ikechukwu CF, Obri AI: Histological changes in the pancreas following administration of ethanolic extract of *Alchornea cordifolia* leaf in alloxan-induced diabetic wistar rats. Nig J Physiol Sci, 24:153–5. 2009.

El-Tantaway WH, Hassanin LA: Hypoglycemic and hypolipidemic effects of alcoholic extract of *Tribulus alatus* in streptozotocin induced diabetic rats. A comparative study with *T. terrestris*. Indian J Exp Biol, 45: 785-790. 2009.

Ezuruike UF, Prieto JM: The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations, J Ethnopharmacol, 155: 857–924. 2014.

Fekam Boyom F, Amvam Zollo PH, Menut C, Lamaty G, Bessière JM: Aromatic plants of tropical Central Africa. Part XXVII. Comparative study of the volatile constituents of five Annonaceae species growing in Cameroon. Flavour Fragr J, 11: 333–338. 1996.

Florence NT, Benoit MZ, Jonas K, Alexandra T, Désiré DDP, Pierre K, Théophile D: Antidiabetic and antioxidant effects of *Annona muricata* (annonaceae), aqueous extract on streptozotocin-induced diabetic rats. J Ethnopharmacol, 151: 784 - 790. 2014.

Gajalakshmi S, Vijayalakshmi S, Devi Rajeswari V: Phytochemical and pharmacological properties of *Annona muricata*: a review. Int J Pharm Sci, 4:13–6. 2012.

Ganesh T, Sen S, Thilagam E, Thamotharan G, Loganathan T, Chakraborty R: Pharmacognostic and anti-hyperglycemic evaluation of *Lantana camara* (L.) var. aculeate leaves in alloxan-induced hyperglycemic rats. Int J Res Pharm Sci, 1(3): 247-252. 2010.

Jiménez VM, Gruschwitz M, Schweiggert RM, Carle R, Esquivel P: Identification of phenolic compounds in soursop (*Annona muricata*) pulp by high-performance liquid chromatography with diode array and electrospray ionization mass spectrometric detection. Food Res Int, 65: 42–46. 2014.

Jiménez VM, Gruschwitz M, Schweiggert RM, Carle R, Esquivel P: Identification of phenolic compounds in soursop (*Annona muricata*) pulp by high-performance liquid chromatography with diode array and electrospray ionization mass spectrometric detection. Food Res Int, 65: 42–46. 2014.

Kanakasabapathi D, Gopalakrishnan VK: Evaluation of antidiabetic potential of aqueous extract of *Passiflora edulis* sims on alloxan induced diabetes mellitus in Wistar albino rats. Int J Pharm Sci Rev Res, 34(1):171-177. 2015.

Kumar PJ, Clark M: Textbook of Clinical Medicine. Pub: Saunders, London, UK. 1099-1121. 2002.

Mallikarjuna RC, Sudeep P, Srinivasan KK: Antidiabetic activities of different extracts of *Caesalpinia bonducella* seed kernels. Pharm Biol, 40: 590-595. 2002.

Maruthupandian A, Mohan VR, Sampathraj R: Antidiabetic, antihyperlipidaemic and antioxidant activity of wattakaka volubilis (l. f) stapf leaves in alloxan induced diabetic rats. Mohan et al., *IJPSR;* Vol. 1 (11): 83-90. 2010.

Matsushige A, Matsunami K, Kotake Y, Otsuka H, Ohta S: Three new megastigmanes from the leaves of *Annona muricata*. J Nat Med, 66, 284–291. 2012.

Ménan H, Banzouzi JT, Hocquette A, Pélissier Y, Blache Y, Koné M, Mallié M, Assi LA, Valentin A: Antiplasmodial activity and cytotoxicity of plants used in west African traditional medicine for the treatment of malaria. J. Ethnopharmacol, 105: 131–136. 2006.

Minari J, Okeke U: Chemopreventive effect of *Annona muricata* on DMBA-induced cell proliferation in the breast tissues of female albino mice. Egypt J Med Hum Genet, 15: 327–334. 2014.

Mishra S, Ahmad S, Kumar N, Sharma BK: *Annona muricata* (the cancer killer): A review. Glob J Pharm Res, 2: 1613–1618. 2013.

Moghadamtousi SZ, Rouhollahi E, Karimian H, Fadaeinasab M, Firoozinia M, Abdulla MA, Kadir HA: The chemopotential effect of *Annona muricata* leaves against azoxymethane-induced colonic aberrant crypt foci in rats and the apoptotic effect of acetogenin annomuricin E in HT-29 cells: A bioassay-guided approach. PLoS ONE, 10. 2015.

Moller DE: New drug targets for type 2 diabetes and the metabolic syndrome. Nature 414: 821–827. 2001.

Muhammad BA, Shamim AQ: *Rauwolfia Serpentina* ameliorates hyperglycemic, haematinic and antioxidant status in alloxan- induced diabetic mice. J Appl Pharm Sci, 3 (07): 136-141. 2013.

National Diabetes Data Group: Diabetes in America. 2nd ed. Bethesda, Md.: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, NIH publication no. 95-1468.1995.

Nawwar M, Ayoub N, Hussein S, Hashim A, El-Sharawy R, Wende K, Harms M, Lindequist U: Flavonol triglycoside and investigation of the antioxidant and cell stimulating activities of *Annona muricata* Linn. Arch Pharm Res, 35: 761–767. 2012.

Osorio E, Arango GJ, Jiménez N, Alzate F, Ruiz G, Gutiérrez D, Paco MA, Giménez A, Robledo S: Antiprotozoal and cytotoxic activities *in vitro* of Colombian annonaceae. J Ethnopharmacol, 111: 630–635. 2007.

Pries E, Garrity L, Kays N, McDaniel P: Growth hormone treatment of a child with growth hormone deficiency, type 1 diabetes and chronic steroid use. J Pediatr Nurs, 21:1-6. 2006.

Rotenstein LS, Kozak BM, Shivers JP, Yarchoan M, Close J, Close KL: The ideal diabetes therapy: what will it look like? How close are we? Clin Diabetes 30: 44–53. 2012.

Rupeshkumar M, Kavitha K, Haldar PK: The role of herbal plants in the diabetes mellitus therapy: an overview. Int J Appl Pharm, 6:1–3. 2014.

Saha D, Ghosh SK, Das T, Mishra SB: Hypoglycemic and antihyperlipidemic effects of *Adiantum caudatum* in alloxan induced diabetes in rats. Asian J Pharm Clin Res, 9:361–3. 2016.

Sandhar P, Kumar HK, Prasher B, Tiwari T, Salhan P, Sharma M: A review of phytochemistry and pharmacology of flavonoids. Int Pharm Sci, 1:25–41. 2011.

Saravanan G, Pari L: Hypoglycaemic and antihyperglycaemic effect of Syzygium cumini bark in streptozotocin-induced diabetic rats. J Pharmacol Toxicol, 3: 1-10. 2008.

Sheehan MT: Current therapeutic options in type 2 diabetes mellitus: a practical approach. Clin Med Res, 1: 189–200. 2003.

Soheil ZM, Mehran F, Sonia N, Gokula M, Hapipah MA, Habsah AK: (2015).*Annona muricata* (Annonaceae): A review of its traditional uses, isolated acetogenins and biological activities. Int J Mol Sci, 16: 15625-15658. 2015.

Sovia E, Ratwita W, Wijayanti D, Novianty DR: Hypoglycemic and hypolipidemic effects of *Annona muricata* L. leaf ethanol extract. Int J Pharm Sci, 9(3):170-174. 2017.

Syed M, Shahid SM, Tabassum M: Effects of diltiazem on electrolytes homeostasis in streptozotocin-induced diabetic rats. Int J Pharmacol, 3(4): 319-329. 2007.

Tanko Y, Mabrouk MA, Adelaiye AB, Fatihu MY, Musa KY: Antidiabetic and some haemotological effects of ethylacetate and nbutanol fractions of *Indigofera pulchra* extract on alloxan- induced diabetic Wistar rats. J Diabetes Endocrinol, 2:1-7. 2011.

Tedong L, Dimo T, Dzeufiet PD, Asongalem E, Sokeng DS, Flejou CP: Antihyperglycemic and renal protective activities of *Anacardium occidentale* leaves in streptozotocin induced diabetic rats. Afr J Tradit Complement Altern Med. 3: 23-35. 2006.

Thang T, Dai D, Hoi T, Ogunwande I: Study on the volatile oil contents of *Annona glabra* L., *Annona squamosa* L., *Annona muricata* L. and *Annona reticulata* L., from Vietnam. Nat Prod Res, 27: 1232–1236. 2013.

Thomas DR: Anemia in diabetic patients. Clin Geriatr Med, 24:529-40. 2008.

Tripoli MG, Guardia ML, Giammanco S, Majo DD: Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review. Food Chem, 104:466–79. 2007.

Vijayameena C, Subhashini G, Loganayagi M, Ramesh B: Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in *Annona muricata*. Int J Curr Microbiol Appl Sci,, 2(1): 1-8. 2013.

Walker R, Whittlesea C: Clinical Pharmacy and Therapeutics. 5th Ed. London: Church hill Livingstone, Elsevier. p. 685. 2012.

Yang C, Gundala SR, Mukkavilli R, Vangala S, Reid MD, Aneja R: Synergistic interactions among flavonoids and acetogenins in graviola (*Annona muricata*) leaves confer protection against prostate cancer. *Carcinogenesis*, 36(6):656-65. doi:10.1093/carcin/bgv1046. 2015.