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# Combined Ethanol Leaf Extract of Sour Sop (Anonna Muricata) and Jackfruit (Artocarpus Heterophyllus) Leaves Amelioratesstreptozotocin Induced Type 2 Diabetes Mellitus In Wistar Albino Rats

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***Abstract:*** *Type 2 diabetes results from the inability of islet cells of the pancreas to produce insulin which regulates blood sugar. A lot of natural products have proven effective in the treatment of diabetes mellitus due to their rich phytochemical contents. Fifty-six(56) rats were given high fat diet (HFD) and 35mg/kg body weight of streptozotocin intraperitoneally to induce type 2 diabetes. The combined effects of A. muricata and A. heterophyllus on the blood glucose levels, amylase, liver function and the kidney function were analysed. The rats were treated with 100 and 200mg/kg body weight of combined ethanol extract of A. muricata and A. Heterophyllus, 200 mg/kg body weight ofethanol extract of A. muricata and A. Heterophyllusuncombined and 50 mg/kg body weight of metformin as a reference drug. The effects of combined ethanol leaf extract of A. muricata and Artocarpus heterophyllus on blood glucose level, liver function and kidney function of wistar albino rats were studied.The result showed an increase in plasma glucose levels and amylase activities of the albino rats (226.33 ± 12.85 mg/dl and 42.97±3.20 u/l respectively) after treatment with HFD and streptozotocin. Treatment of the albino rats with 100 and 200 mg/kg body weight of the combined ethanol leaf extract for 30 days led to a statistically significant decrease in plasma glucose levels of the animals 96.33±2.30 and 86.33±7.51 respectively. A significant decrease ($p<0.05$) was also observed in the AST, ALP and ALT activities of the diabetic after treatment for 30 days 127.33±5.03, 73.33 ± 6.80 and 73.33±6.81u/l respectively.The result of this research indicate that combined ethanol leaf extract of A. muricata and A. Heterophyllus has a synergetic effect on streptozotocin induced type 2 diabetes mellitus on wistar albino rats.*

***Key Words:*** *A. muricata,A. Heterophyllus, streptozotocin, diabetes mellitus, metformin, synergestic*

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## I. Introduction

Advancement in medical technology has led to the production of different kinds of drugs which has helped in the cure and prevention of different kinds of diseases and infections. Before thistime traditional medicine was practiced and accepted in Africa and has been very helpful in the treatment of diseases and infections. Due to the negative side effect caused by the use of some orthodox medicines a lot of interest has been focused on natural products especially in Africa. There has been an increase in the intake of fruits and vegetables due to the awareness created by researchers on their rich content of chemicals otherwise known as phytochemicals. More than 500 species of plant exist [1]and they are rich in bioactive chemicals which make them effective in the treatment of different kinds of diseases and infections. A. muricata is one of such plants which have been used over the years locally. It is an edible fruit tree which is found growing almost everywhere in Nigeria. The fruit is sweet to test and rich in minerals and vitamins. The back of the fruit is spiky and not edible but is often consumed by herbivores. The leaves are greenish in colour and not eaten as vegetables. Decoctions made from the leaves are used in traditional medicine to treat fever and to reduce blood sugar levels.It is rich in phytochemicals which has antioxidant activities. Studies show that the leaves of A. muricatacontains a compound known as Annohexocin a mono-THF annonaceous acetogenin which is responsible for its anticancer activities especially in animal models[2,3].Earlier studies have shown that the leaves of A. muricata contains rich deposits of phytochemicals including phenolic compounds, tannins saponines, phytosterol, terpenoids, anthroquinones, flavonoids, alkaloids and others.[3,4] The leaf extract of sour sop have other biological activities including antibacterial activities attributed to its phytochemical content [5], Larvicidal activity against mosquito larva, A. aegypti specie,[6] antiviral and antimicrobial activity [7]. Ethnobotanical uses of sour sop leaves has also been reported, it is used locally as a sedative. It is also used locally to treat hypertension, rheumatism, liver diseases, diabetes and inflammations.[8, 9]

Antidiabetic and antihypertensive effect of sour sop has also been reported, this effect was attributed to its high content of phenolic compound.[10] Brewed tea of sour sop leaves has inhibitory effect against α-glycosidase enzyme; this effect is due to the high tannin, flavonoids and triterpenoid content of the leaves [11]. Florenceet al., 2014 demonstrated antidiabetic and antilipidemic effect of aqueous extract of A. muricata leaves in streptozotocin induced diabetic rats.[12]

Jackfruit (Artocarpus heterophyllus Lam.) is a fruit tree which belongs to the family of Moraceae.[13] It is a tropical plant found growing mainly in the southern area of Asia.[13] It produces a very large fruit which is about the biggest fruit existing.[14]The leaves of jack fruit are rich in phytochemicals which are responsible for many of its pharmacological activities. Plant chemicals such as sapogenins, cycloartenone, cycloartenol, β-sitosterol, flavonoids and tannins have been identified in the leaves of A. heterophyllus[11,15].The antioxidant activities of jack fruit leaves has been reported, diphenylpicrylhydrazyl radical scavenging activity, reduced lipid peroxidation activities and $Fe^{+2}$ chelating activity of the leaf extract of A. heterophyllus in vitro have been linked to its flavonoids content.[15]Ethno pharmacological claims show that the leaves of jackfruit are used in the treatment of hypertension, diabetes, fever, boils, wounds and skin diseases[13,14]. Animal studies have shown that the leaves of A. heterophyllus have different pharmacological activities including anticonvulsant activity, antiulcer activity, antidiabetic and antilipidemic activity.[15,16,17]

Diabetes mellitus is an age long metabolic disorder which affects a great number of people worldwide. Different types of diabetes mellitus have been identified but type 2 diabetes mellitus remains the most prevalence. A lot of efforts has been made by researchers to produce drugs that can cure diabetes but it has remained incurable and can only be managed by proper dieting and constant exercise especially type 2 diabetes mellitus while type 1diabetes mellitus can be managed by constant injection of insulin. Type 2 diabetes is due to impaired insulin secretion resulting to elevated blood sugar levels.

## II. Materials and methods

**2.1 Plant materials and reagents**

The leaves of A. muricata were obtained from Abuja park of University of Port Harcourt while the leaves of A. heterophyllus were obtained from Ozuoba Obior/Akpo Local Government Area of Rivers State, Nigeria.The botanical identity of Artocarpus heterophyllus and Anonna muricate were confirmed in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The voucher specimen of the plants have been deposited at the habarium of Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria

**2.2Preparation of Extract**

A. muricata and A. heterophyllus Extract: The leaves of A. murica and A. heterophyllus were washed and shade dried, after which the leaf powder was prepared using home grinder/blender. About 200g of the powdered A. murica and A. heterophyllus were weighed and soaked in 1000ml of 95% Ethanol for 48 hours after which they were sieved using a muslin cloth and afterwards filtered with Whatmann paper size 1. The filtrate was concentrated using Rotary Evaporator at 45$^{o}$ C, the weight of the concentrates was taken and the percentage yield calculated. The extracts were kept at 4$^{o}$ C until usage.

**2.3Drugs and chemicals**

Streptozotocin (STZ) was purchased from Sigma Chemicals Co. St. Louis, MO, USA. STZ solution was freshly prepared each time of use in cold citrate buffer 0.1 (pH 4.5). Metformin was obtained from E-blend Pharmaceutical, Abuja Park University of Port Harcourt, Rivers State Nigeria.Glucose, Amylase, AST, ALT and ALP activities, total bilirubin, conjugated bilirubin, total protein,serum albumin,urea and creatinine levels were analysed used randox kit (Randox laboratories Ltd, United Kingdom)

**2.4 Equipments**

ISO 4000 Automated electrolyte analyser. SFRI, France;Ultra-low temperature deep Freezer (-80$^{o}$C) (Haier Thermocool);Electrothermal Water Bath SM 801A UNISCOPE (Surgifriend England); Automatic Biochemistry Analyzer (BURACHEM-3) BAUR; Water Distiller A Pure-981 ES Germany.

**2.5 Toxicity test for streptozotocin**

Toxicity test was conducted using nine (9) rats. They were grouped into three (3) and each group contained three (3) rats which were marked for easy identification. The rats were fasted overnight and were given different doses of streptozotocin intraperitoneally. The first group was given 30mg/kg body weight of streptozotocin, the second group was given 40mg/kg body weight of streptozotocin and the third group was given 50m/kg body weight of streptozotocin.

**2.6Experimental Animals**

About 56 male wistar albino rats weighing 150 to 200g were used for this experiment. The animals were kept understandard laboratory condition (12 h light/darkness; at 25 ± 3$^{o}$C) with standard animal diet and water available ad libitum. The study was approved by the ethical committee of the institute.

**2.6.1 Experimental induction of type 2 diabetes**

After acclimatization, all animals except normal control were allowed free access of high-fat diet 20% SUCROSE, 10% margarine (baking fat), 2.5 % egg yolk and 67.5 % finisher (animal feed). After 21 days of high-fat diet (HFD), overnight- fasted (12 h) rats were administered a single injection of freshly prepared STZ (35 mg/kg, i.p. in 0.1 M citrate-phosphate buffer, pH 4.5). Blood was withdrawn from the tail vein 72 h after STZ treatment and assessed for hyperglycemia by measuring fasting serum glucose (FSG) level. The rats with FSG level above 13.89 mmol/l were considered diabetic and included in the study

**2.6.2Experimental design for anti-diabeticeffects**

Research was carried out according to the rules and regulation guiding the use of animals. A total of fifty-six rats were used for the experiment. They were sorted into five groups with eight (8) animals each. Mortality rate was observed and the animals were grouped as follows:

Group 1: Normal control, non-diabetic.

Groups 2: Diabetes control (35mg/kg body weight of Streptozotocin)

Group 3: Diabetes + metformin (50mg/kg body weight of metformin)

Group 4: Diabetes + 100mg/kg body of combined ethanol extract of A. muricata and A. heterophyllus

Group 5: Diabetes+ 200mg/kg body of combined ethanol leaf extract of A. muricata and A. heterophyllus

Group 6: Diabetes + 200 mg/kg A. muricata and 200 mg/kg body of ethanol leaf extract of A. muricata

Group 7: Diabetes + 200 mg/kg A. heterophyllusand 200 mg/kg body of ethanol leaf extract of A. heterophyllus

Treatment started 72 hours after induction of type 2 diabetes mellitus. Wistar albino rats whose blood glucose level were up to 200mg/dl where selected for the study. The animals were sacrificed at 15 days interval.

**2.6.3** Estimation of biochemical parameters

The biochemical parameters were analysed using standard procedures. Serum electrolyte: The electrolyte (Na, K, $HCO^-_3$, Ca, and Cl) level in the rat serum was analysed using ISO 4000 Automated electrolyte analyser.

**2.8Statistical analysis**

All data were subjected to statistical analyses. Values are reported as Mean ± standard error of mean (SEM) while one way ANOVA was used to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 16. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($P<0.05$)**3.0 Result**

## III. Results

**3.1 Effect of combined ethanol leaf extract ofA. muricata and A. heterophyllus on blood glucose level and amylase activities**

Table 1 below shows the effect of combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight) on the glucose concentration of type 2 diabeticrats. A statistically significant increase ($p<0.05$) was observed in the glucose concentration of Group 2 rats (Diabetes control) when compared with Group 1 (normal control) and 3 (diabetes + metformin) after 15 and days treatment. A significant decrease ($p<0.05$) was observed in the serum glucose concentration of Group 4, 5, 6 and 7 rats when compared with group 2 after 15 days and 30 days treatment. The serum amylase activities of the rats are also shown in table 1 below; a significant increase ($p<0.05$) was observed in the amylase activity of Group 2rats when compared with Group 1rats after 15 and 30 days treatment. A decrease ($p<0.05$) was observed in the amylase activities of Group 4 and Group 5 rats when compared with theGroup 2rats 15 days after treatment. Adecrease ($p<0.05$) was observed in the serum amylase activities of the group 4 and 5 ratsafter treatment for 15 days while a statistically significant increase was observed in the amylase activity of Group 6 and 7 after treatment for 30 days.

**3.2. Effect of combined ethanol extract of A. muricata and A. heterophyllus on the AST, ALT and ALP activities of type 2 diabetic wistar albino rats**

Table 2 below shows the effect of combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight) on the AST activity of type 2 diabetic wistar albino rats. A significant increase ($p<0.05$) was observed in the AST activity of the group 2 rats when compared to all the other groups 15 and 30 days after treatment. The result shows a statistically significant decreases ($p<0.05$) in the AST activities of Group 3, 4 and 5, 6 and 7 after treatment for15 and 30 days after treatment. A statistically significant increase ($p<0.05$) was observed in the alkaline phosphatase (ALP) activity of the group 2wistar albino rats 15 and 30 days after treatment whereas a statistically significant decrease ($p<0.05$) was observed in the alkaline phosphatase (ALP) activity of group 3, 4, 5, 6 and 7 rats 15 and 30 days after treatment. A significant increase ($p<0.05$) was observed in the ALT activity of the Group 2 animals when compared with all

the groups after treatment whereas a decrease ($p<0.05$) was observed in the ALT activities of the various Groups after treatment for 15 and 30 days.

**3.3. Effect of combined ethanol extract of A. muricata and A. heterophyllus on the total bilirubin, conjugated bilirubin, protein and albumin levels of type 2 diabetic rats**

Table 3 below shows the effect of combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight), on the serum total bilirubin levels of type 2 diabetic rats. A statistically significant ($p<0.05$) increase was observed in the total bilirubin level of the Group 2 rats. Treatment with combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight) caused a significant decrease ($p<0.05$) in the serum total bilirubin levels of Group 4, 5, 6 and rats respectively after treatment for 30 days. The result also showed a statistically significant decrease ($p<0.05$) in the serum total bilirubin level of Group 3 rats after treatment for 15 and 30 days.

There was an increase ($p<0.05$) in the serum conjugated bilirubin level of the Group 2 rats after 15 and 30 days as shown in table 2 below. Treatment with combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight), caused a significant decrease ($p<0.05$) in the conjugated bilirubin levels of Group 4, 5, 6 and 7 animals after treatment for 15 and 30 days. The result also showed a decrease ($p<0.05$) in the serum conjugated bilirubin level of Group 3 rats.

The effect of combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight) on the serum total protein levels of type 2 diabetic wistar albino rats was studied. No significant ($p<0.05$) effect was observed 15 days after various treatment, but an increase was observed in the total protein levels of the group 2 rats. A Treatment with metformin and combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight), ethanol extract of A. muricata and A. heterophyllus(200mg/kg body weight) caused a statistically significant decrease ($p<0.05$) in the total protein levels of Group 3, 4, 5, 6 and 7 animals respectively. A slight increase was observed in the albumin concentration of the group 2 rats but not statistically significant ($p<0.05$) after treatment for 15 and 30 days.

**3.4. Effect of combined ethanol extract of A. muricate andA. heterophyllus on the urea and creatinine levels of the type 2 diabetic wistar albino rats**

The effect of combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight) on the urea levels of type 2 diabetic rats is as shown in table 4 below. An increase which is significant ($p<0.05$) was observed in the urea levels of the group 2 rats 15 and 30 days after treatment. Treatment with combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight), etganol extract ofA. muricata and A. heterophyllus (200mg/kg body)caused a statistically significant decrease in the urea levels of group 4, 5, 6 and 7 rats respectively after treatment for 15 and 30 days.

Result from the present research shows a slight increase in the serum creatinine levels of the group 2 rats after treatment for 15 days. Asignificant increase ($p<0.05$) was observed in the creatinine levels of the group 2 rats after 30 days when compare with group 1 (normal control) and group 3 (drug control) rats. A decrease was observed in the serum creatinine levels of group 3, group 4 and group 5 rats after treatment with metformin, 100 and 200 mg/kg body weight of combined extract of A. muricata and A. heterophyllus respectively but not statistically significant ($p<0.05$) after treatment for 15 and 30 days.

**3.5. Effect of combined ethanol extract of A. muricata and A. heterophyllus on the serum electrolyte concentration of type 2 diabetic wistar albino rats**

Table 5 below shows the effect of combined ethanol extract of A. muricata and A. heterophyllus (100mg/kg body weight and 200mg/kg body weight) on the serum electrolyte concentration of type 2 diabetic rats. A slight decrease was observed in the serum sodium concentration of groups 4 and 5 but not statistically significant ($p<0.05$) when compared with group 1 and 2. The result of this research showed no significant ($p<0.05$) effect on the serum potassium, calcium, chloride and bicarbonate concentration of the rats in all the groups after treatment for 15 and 30 days.

**Table 1:The effect combined ethanol extract of A. muricata and A. heterophyllus glucose and amylase activity**

| | Glucose (mg/dl) | | Amylase (u/l) | |
|---|---|---|---|---|
| **Groups** | **After 15 days** | **After 30 days** | **After 15days** | **After 30 days** |
| **Normal control** | $62.33\pm12.89^{b}$ | $77 \pm 5.00^{b}$ | $22.30\pm2.45^{bc}$ | $21.07\pm2.10^{bc}$ |
| **Diabetes control** | $232\pm27.78^{ac}$ | $226.33 \pm 12.85^{ac}$ | $44.60\pm2.85^{a}$ | $42.97\pm3.20^{a}$ |
| **Diabetes+ Metformin** | $99.33\pm1.53^{b}$ | $83.33 \pm 12.06^{b}$ | $35.40\pm4.03^{a}$ | $34.10\pm3.04^{a}$ |
| **Diabetes + 100mg combined Extract** | $65.00\pm9.849^{b}$ | $96.33\pm2.30^{b}$ | $30.73\pm3.88^{b}$ | $27.83\pm4.47$ |

| | | | | |
|---|---|---|---|---|
| **Diabetes + 200mg combined Extract** | 120.33±7.02[ab] | 86.33±7.51[b] | 31.23±3.84[b] | 32.47±2.52 |
| **Diabete+ 200mg A. muricata** | 136.66±17.95[ab] | 93.33±3.05[b] | 37.43±7.57[a] | 34.40±4.20[b] |
| **Diabetes + 200mg A. Heterophyllus** | 132.33±29.16[ab] | 98.66±5.03[b] | 35.27±1.84[a] | 34.80±7.24[b] |

All values are expressed as mean ±S.D. (n = 4/group).
Superscript "a" shows significant difference, ($p<0.05$) when Normal control (NC) is compared with other groups.
Superscript "b" shows significant difference, ($p<0.05$) when Disease control (DC) is compared with other groups.
Superscript "c" shows significant difference, ($p<0.05$) when Metformin Drug control (MDC) is compared with other groups.

**Table 2: The effect combined ethanol extract of A. muricata and A. heterophyllus liver enzymes**

| **Groups** | **AST (u/l)** | | **ALP(u/l)** | | **ALT (u/l)** | |
|---|---|---|---|---|---|---|
| | **Day 15** | **Day 30** | **Day 15** | **Day 30** | **Day 15** | **Day 30** |
| **Normal control** | 123.66±3.51[b] | 124.66±5.68[b] | 61.00 ± 6.08[b] | 57.33±9.07[b] | 61.00±6.08[b] | 57.33±9.07b |
| **Diabetes control** | 218.66±19.60[ac] | 235±5.56[ac] | 128.33±6.02[ac] | 134.66 ± 2.51[ac] | 128.33±6.03[abc] | 134.67±2.52[abc] |
| **Diabetes + Metformin** | 124.33±23.02[b] | 131±10.58[b] | 52.33±3.21[b] | 46.33±12.42[b] | 52.33±3.21[bc] | 46.33±12.42[bc] |
| **Diabetes + 100mg combined Extract** | 125.00±26.21[b] | 135.33±11.37[b] | 89.33±9.07[b] | 81.00±6.25[b] | 89.33±9.07[a bc] | 81.00±6.24[abc] |
| **Diabetes + 200mg combined Extract** | 127.33±7.50[b] | 127.33±5.03[b] | 90.33±9.45[b] | 73.33 ± 6.80[b] | 90.33±9.45[a bc] | 73.33±6.81[bc] |
| **Diabete+ 200mg A. muricata** | 135±30.04[b] | 134.66±7.51[b] | 90.33±9.45[b] | 73.33 ±6.80[b] | 76.67±16.80[b] | 73.33±5.03[bc] |
| **Diabetes + 200mg A. Heterophyllus** | 129±9.00[b] | 127±3.61[b] | 76.66±16.80[b] | 73.33±5.03[b] | 78.00±8.89[b] | 72.00±4.00[bc] |

All values are expressed as mean ±S.D. (n = 4/group).
Superscript "a" shows significant difference, ($p<0.05$) when Normal control (NC) is compared with other groups.
Superscript "b" shows significant difference, ($p<0.05$) when Disease control (DC) is compared with other groups.
Superscript "c" shows significant difference, ($p<0.05$) when Metformin Drug control (MDC) is compared with other groups.

**Table 3:The effect combined ethanol extract of A. muricata and A. heterophyllus on total bilirubin, conjugated bilirubin, total protein and albumin levels of the wistar albino rats**

| **Groups** | **Total Bilirubin (µmol/l)** | | **Conjugated Bilirubin (µmol/l)** | | **Total Protein(g/l)** | | **Albumin (g/l)** | |
|---|---|---|---|---|---|---|---|---|
| | Day 15 | Day 30 | Day 15 | Day 30 | Day 15 | Day 30 | Day 15 | Day 30 |
| **Normal control** | 19.33±4.16[b] | 18.00±3.00[b] | 13.57±3.67[b] | 15.70±2.45[b] | 49.33±6.03 | 43.67±9.07[b] | 28.67±2.08 | 27.67±3.06 |
| **Diabetes control** | 32.67±5.69[abc] | 38.33±2.52ac | 24.83±1.86[ac] | 25.17±2.70[a c] | 66.00±6.08 | 73.00±1.00[ac] | 32.33±2.08 | 32.67±2.08 |
| **Diabetes + Metformin** | 19.33±4.16[b] | 16.00±2.00[bc] | 10.53±2.55[b] | 10.80±2.02[a b] | 53.00±1.73 | 53.33±8.08[b] | 26.33±5.03 | 27.33±3.06 |
| **Diabetes + 100mg combined Extract** | 16.00±2.00[abc] | 19.00±2.00[abc] | 10.60±2.59[b] | 11.33±3.00[b] | 54.00±6.56 | 52.33±4.51[b] | 27.00±3.61 | 27.00±2.65 |
| **Diabetes + 200mg combined Extract** | 19.33±3.06[ab] | 22.67±3.06[bc] | 10.97±1.69[b] | 10.33±1.21[b] | 51.33±9.07 | 53.00±4.58[b] | 26.67±2.08 | 27.00±3.00 |
| **Diabete+** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **200mg A. muricata** | 22.67±3.06[b] | 17.67±3.51b[c] | 11.20±2.43[b] | 10.10±2.10[b] | 51.33±4.16 | 55.00±2.65[b] | 26.67±2.08 | 26.00±3.00 |
| **Diabetes + 200mg A. Heterophyllus** | 18.67±3.06[b] | 18.67±.58[bc] | 12.53±2.51[b] | 11.10±1.04[b] | 59.00±7.00 | 53.00±4.36[b] | 28.00±2.00 | 25.33±3.51 |

All values are expressed as mean ±S.D. (n = 4/group).
Superscript "a" shows significant difference, ($p<0.05$) when Normal control (NC) is compared with other groups.
Superscript "b" shows significant difference, ($p<0.05$) when Disease control (DC) is compared with other groups.
Superscript "c" shows significant difference, ($p<0.05$) when Metformin Drug control (MDC) is compared with other groups.

**Table 4** showing the effect combined ethanol extract of A. muricata and A. heterophyllus on urea and creatinine levels of the wistar albino rats

| | **UREA(Mmol/l)** | | **CREATININE (Mmol/l)** | |
|---|---|---|---|---|
| **Groups** | Day 15 | Day 30 | Day 15 | Day 30 |
| **Normal control** | 11.07±.74[b] | 11.17±1.03[b] | 173.33±3.79 | 168.67±4.16[b] |
| **Diabetes control** | 39.33±5.62[ac] | 44.23±2.76[ac] | 186.67±5.03 | 189.00±2.00[ac] |
| **Diabetes + Metformin** | 4.77±1.55[b] | 6.03±1.05[b] | 175.67±1.15 | 171.67±4.51[b] |
| **Diabetes + 100mg combined Extract** | 7.00±0.89[b] | 5.90±2.95[b] | 175.67±10.12 | 180.33±7.64 |
| **Diabetes + 200mg combined Extract** | 6.63±.38[b] | 7.57±.97[b] | 175.67±10.97 | 177.00±3.61 |
| **Diabete+ 200mg A. muricata** | 7.00±4.14[b] | 7.87±1.70[b] | 175.33±5.13 | 173.00±3.61[b] |
| **Diabetes + 200mg A. Heterophyllus** | 6.13±.61[b] | 7.40±2.89[b] | 170.67±3.51 | 167.00±9.64[b] |

All values are expressed as mean ±S.D. (n = 4/group).
Superscript "a" shows significant difference, ($p<0.05$) when Normal control (NC) is compared with other groups.
Superscript "b" shows significant difference, ($p<0.05$) when Disease control (DC) is compared with other groups.
Superscript "c" shows significant difference, ($p<0.05$) when Metformin Drug control (MDC) is compared with other groups.

**Table 5:The effect combined ethanol extract of A. muricata and A. heterophyllus serum sodium, potassium and bicarbonate levels of the wistar albino rats**

| | **Sodium (Mmol/l)** | | **Potassium(Mmol/l)** | | **Bicarbonate (Mmol/l)** | |
|---|---|---|---|---|---|---|
| **Groups** | **Day 15** | **Day 30** | **Day 15** | **Day 30** | **Day 15** | Day 30 |
| **Normal control** | 128.67±6.81 | 128.67±5.13[b] | 3.47±.06[b] | 3.47±.55[b] | 27.00±2.65 | 23.00±2.00 |
| **Diabetes control** | 149.67±6.43 | 151.33±11.59[a] | 5.63±.51[ac] | 5.80±.10[ac] | 33.00±2.65 | 31.33±7.64 |
| **Diabetes + Metformin** | 138.00±2.65 | 136.33±2.08 | 3.83±.35[b] | 3.30±.72[b] | 26.67±1.15 | 25.00±3.61 |
| **Diabetes + 100mg combined Extract** | 135.33±12.50 | 135.33±1.53 | 4.30±.53 | 4.17±.25[a] | 26.67±3.06 | 26.33±2.08 |
| **Diabetes + 200mg combined Extract** | 139.33±7.37 | 141.00±6.56 | 4.20±.70[b] | 4.20±.46[a] | 23.00±1.00[b] | 25.33±3.79 |
| **Diabete+ 200mg A. muricata** | 140.67±4.04 | 136.67±8.02 | 3.50±.26[b] | 3.70±.26[a] | 24.00±3.46[b] | 24.67±2.52 |
| **Diabetes + 200mg A. Heterophyllus** | 136.67±11.85 | 142.00±4.00 | 3.47±.61[b] | 3.23±.15[a] | 29.33±1.15 | 28.00±2.00 |

All values are expressed as mean ±S.D. (n = 4/group).
Superscript "a" shows significant difference, ($p<0.05$) when Normal control (NC) is compared with other groups.
Superscript "b" shows significant difference, ($p<0.05$) when Disease control (DC) is compared with other groups.
Superscript "c" shows significant difference, ($p<0.05$) when Metformin Drug control (MDC) is compared with other groups.

**Table 6:The effect combined ethanol extract of A. muricata and A. heterophyllus serum calcium and chloride levels of the wistar albino rats**

| | Calcium (Mmol/l) | | Chloride (Mmol/l) | |
|---|---|---|---|---|
| **Groups** | **Day 15** | **Day 30** | **Day 15** | **Day 30** |
| **Normal control** | 1.07±1.03 | 1.07±0.45 | 72.33±4.62 | 68.00±7.94[b] |
| **Diabetes control** | 1.47±00.42 | 1.60±0.46 | 82.67±5.51 | 86.67±4.51[a] |
| **Diabetes+ Metformin** | 1.71±.52 | 1.26±0.14 | 75.00±3.00 | 73.00±2.00 |
| **Diabetes + 100mg combined Extract** | 1.71±0.46 | 1.90±0.19 | 80.67±15.04 | 77.00±6.56 |
| **Diabetes + 200mg combined Extract** | 1.83±0.67 | 1.83±0.51 | 83.67±13.05 | 85.00±8.19 |
| **Diabete+ 200mg A. muricata** | 1.72±0.33 | 1.64±0.56 | 83.67±6.81 | 80.33±8.08 |
| **Diabetes + 200mg A. Heterophyllus** | 1.53±0.37 | 1.34±0.26 | 77.67±4.93 | 77.67±6.66 |

All values are expressed as mean ±S.D. (n = 4/group).
Superscript "a" shows significant difference, ($p<0.05$) when Normal control (NC) is compared with other groups.
Superscript "b" shows significant difference, ($p<0.05$) when Disease control (DC) is compared with other groups.
Superscript "c" shows significant difference, ($p<0.05$) when Metformin Drug control (MDC) is compared with other groups.

3.6. **Histopathological findings**

The effect of HFD-STZ and combined ethanol leaf extract of A. muricata and A. heterophyllus on the pancreatic tissue of the rats are as shown in the plates A-E below. Haematoxylin and Eosin stained sections from the control group 1 (plate A), shows a normal histological structure of the pancreatic tissue with lobules of pancreatic acini which is separated from each other by interlobular connective tissues. The photomicrograph also shows the islets of Langerhans which appeared as a pale stained rounded or oval areas surrounded by the pancreatic acini.

Haematoxylin and Eosin stained section from diabetes control group 2 (plate B) shows that STZ caused marked morphological changes in the pancreatic tissue structure. The architecture of the islet of Langerhans is totally distorted with few islet cells and vacuoles.

Haematoxylin and Eosin stained sections from the control group 3 (plate C) shows a restored pancreatic islet with pancreatic islet cells and a distinctive border between exocrine and endocrine portions of the pancreatic tissue.

Haematoxylin and Eosin stained sections from the control group 4 (plate D) shows a restored pancreatic islet surrounded by pancreatic acini with a distinctive intra and interlobular connective tissues. Numerous islet cells can also be observed in the islet of Langerhans.

Haematoxylin and Eosin stained sections from the control group 5 (plate E) shows a restored pancreatic islet with numerous islet cells. The islet of Langerhans is surrounded by lobulated pancreatic acini. A distinctive border between the exocrine and endocrine portions of the pancreatic tissue is observed.Heamatoxylene and Eosin stained sections from the control group 6 (plate F) shows small clusters of newly formed pancreatic islet with no distinctive border between the exocrine and endocrine portion of the pancreatic tissue. A lot of intra lobular connective tissues are observed with pancreatic acini separated by interlobular connective tissues

Heamatoxylene and Eosin stained sections from the control group 7 (plate G) showing newly formed pancreatic islets. Distinctive pancreatic islets are seen with pancreatic cells.

**3.6.1 Photomicrograph of the pancreas**

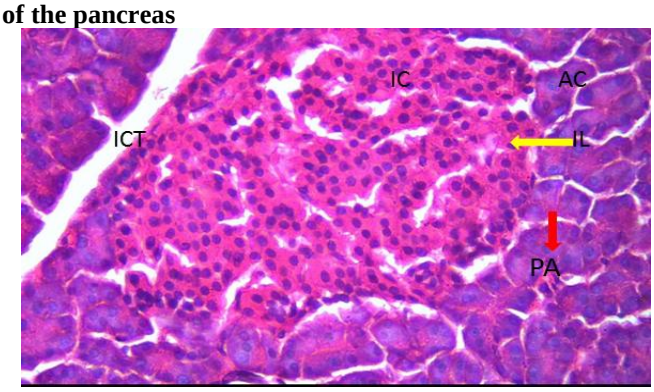


Plate A:The pancreas of Group 1 rats (x400)

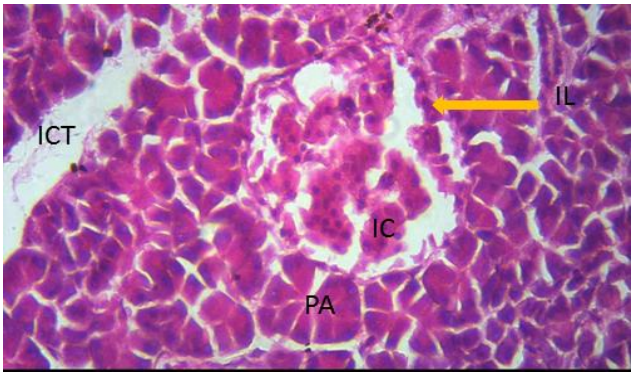


Plate B:The pancreas of Group 2 rats(x400)

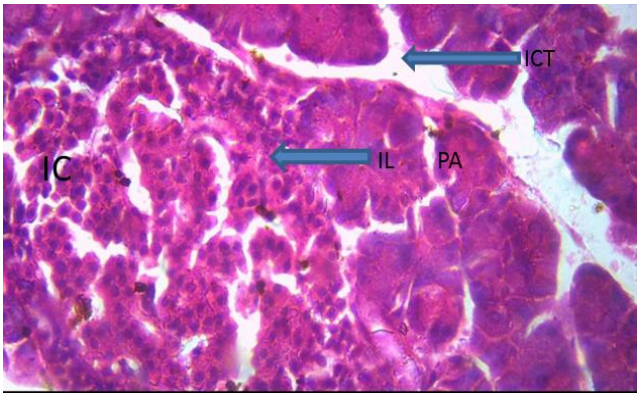


Plate C:The pancreas of Group 3 rats(x400)

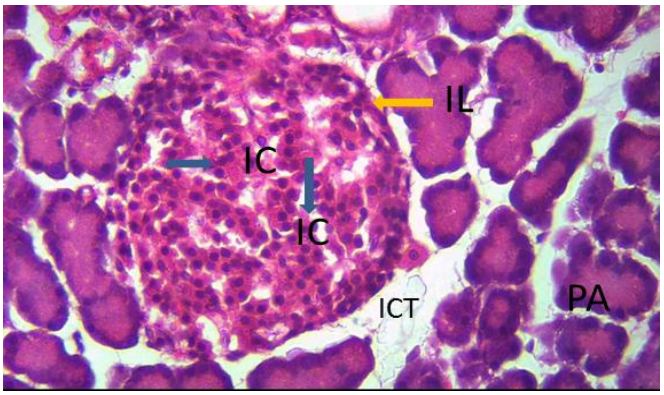


Plate D:The pancreas of Group 4 rats(x400)

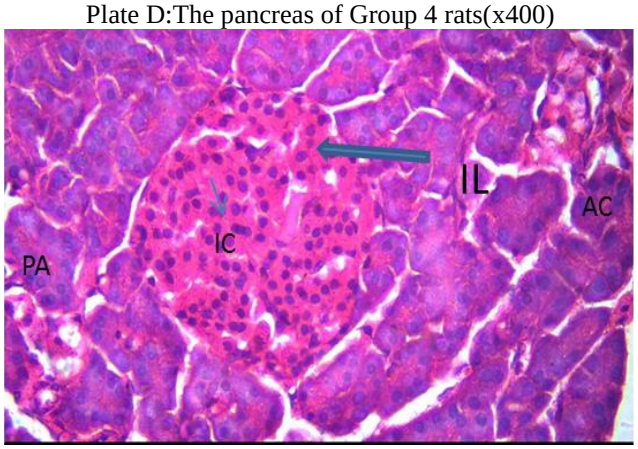


Plate E showing the pancreas of Group 5 rats (x400)

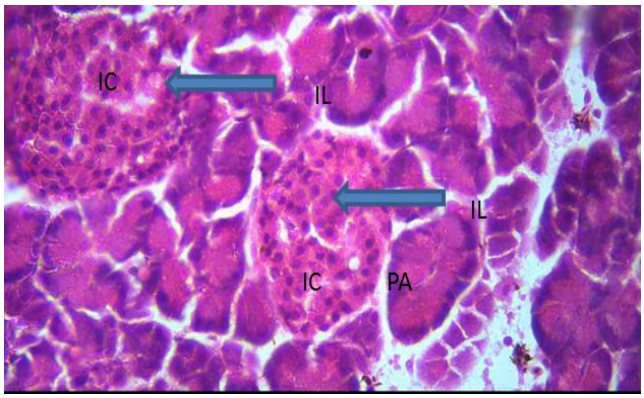


Plate F:The pancreas of Group rats(x400)

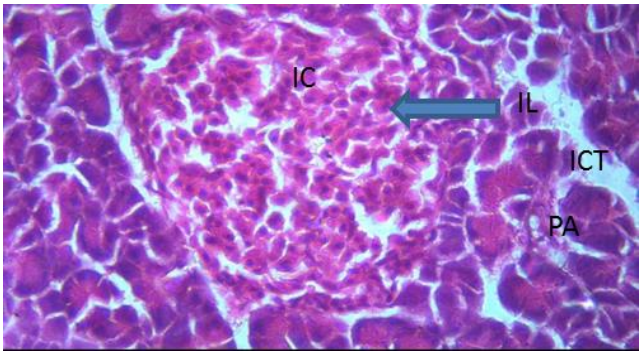


Plate G:The pancreas of Group 7 rats(x400)

**Images are representative of three animals per experimental group.**

## IV. Discussions

Type 2 diabetes mellitus results from defect in insulin responsiveness in the presence of high glucose level after meal. These results in reduced uptake of glucose to the membranes hence increased blood glucose level. The use of herbs in the treatment of different kinds of disease has been in the fore front of research for some time now. Many of these plants are rich in plant chemicals which have been used in the treatment of different kinds of diseases.A lot of plants have proven effective in the treatment of diabetes both in human and animal studies. In the present study, the antidiabetic effect of combined ethanol leaf extract of A.muricata and A. heterophyllus were investigated. Treatment with A. muricata and A. heterophyllus caused a significant hypoglycemic effect in high fat diet and streptozotocin induced type 2 diabetes. The hypoglycemic effect of the combinedtherapy could be as a result of the high phytochemical content of the plants. Chackrewarthy and Thabrew, 2012, demonstrated antidiabetic effect of ethyl acetate fraction of A. heterophyllus leaves whileChandrika et al.,2006,demonstrated the ant diabetic effect of flavonoid fractions of A. heterophyllus leaves.[18,19]Florence et al., 2013 also demonstrated the anti-diabetic effect of aqueous extract of A. muricata. Streptozotocin acts by destroying the pancreatic β-cell.[12] It is possible that the anti-diabetic effect of the combined extracts may be due to their ability to restructure the pancreatic β-cell for insulin production; this could be as a result of the high phytochemical content of the plants. The hypoglycemic effect could also be due to the presence of phytol in both plants as seen in our previous study (unpublished). Phytol a diterpene, increases insulin responsiveness and glucose uptake.[20]Amylase is an exocrine enzyme whose main function is digestion. They are secreted both in the salivary gland and the pancreas. Research has shown that the presence of amylase in the serum is related to endocrine and metabolic disorders.[21] Elevated serum activities have been linked to acute pancreatitis, diabetic ketoacidosis and kidney dysfunction.[22]To further validate the anti-diabetic and β-cell-preserving activity of A. muricata and A. heterophyllus in HFD-STZ-induced diabetic rats, heamatoxylene and eosin staining of the pancreatic tissues were performed. Microscopic examination of the stained pancreas tissues revealed distoreted pancreatic islet indicating β-cell failure and damage in HFD-STZ treated rats.

Liver diseases are common in diabetic patients; they include liver cirrrohsis, non-alcoholic fatty liver disease which may lead to elevated liver enzyme activities.[23]In the present studies, a statistically significant increase ($p<0.05$) was observed in the liver enzymes of streptozotocin treated groups. The leakage of these enzymes (ALT, AST and ALP) into the blood may result from liver damage as part of complications of type 2 diabetes mellitus.Mathur et al., 2016 suggested that elevated ALP may be due to cholestasis resulting from dysregulation in the metabolism of fat.[24] This implies that elevated alkaline phosphatase enzyme(ALP) in type 2 diabetes may not be due to liver damage but cholestasis. Alanine amino transaminase (ALT) is involved in gluconeogenesis, ALT catalyses the transamination of L-alanine and α-ketoglutarate to form pyruvate and L-glutamate. Type 2 diabetes mellitus increases gluconeogenesis due to increased supply of alanine and thus increased conversion of alanine to glucose. Hence there is up regulation of ALT in type 2diabetes mellitus which leads to its leakage in to the blood. Research has also shown that elevated Aspartate amino transaminase activities (AST) might be observed in type 2 diabetes, this has been linked to lipid peroxidation, peroxisomal beta-oxidation, toxin formation in the liver and probably due to inflammatory cells.[24] Though, animal researches in diabetes have shown incidence of liver damage, in animal studies it is not clear whether liver damage is as a result of the drugs used in inducing diabetes or as a result of complications arising from the disease itself.[25] Treatment with combined ethanol extract of A. muricata and A. heterophyllus resulted in the reduction of ALT, AST and ALP activities. This reduction can be attributed to the high phytochemical contents of the plants.

Bilirubin is the end product of heme metabolism, once produced they are transported to the liver where they are conjugated to a water soluble form for easy excretion by uridine diphosphate–glucuronosyl transferase (UDP-GT) (Hull and Agarwal, 2014). Elevated total and conjugated bilirubin in serum is as a result of defect in the excretion of bilirubin arising from liver damage and or haemolysis. Result of this research showed an elevated total and conjugated bilirubin levels in the streptozotocin induced type 2 diabetic animals. Treatment with combined ethanol extract of A. muricata and A. heterophyllus caused a marked decrease in the total and conjugated bilirubin levels of the treated animals. This suggest that the combined ethanol extract A. muricata and A. heterophyllus contains plant chemical which has the potential to reduce the elevated total and conjugated bilirubin levels.

Albumin is present in the plasma in a large quantity and it is highly glycated in diabetes mellitus. Various researches suggest that reduced serum albumin levels in diabetes could be linked to increased plasma protein glycation.[26,27] Elevated albumin levels in the blood of the group 2 (diabetes control) rats can be linked to dehydration which is common in diabetes or liver disease. These effects were altered by the administration of combined ethanol extract ofA. muricata and A. heterophyllus

Urea and creatinine are both waste products that are excreted by the kidney. They serve as markers for kidney damage. Kidney failure or nephropathy is one of the complications of diabetes mellitus. Urea and creatinine are passed into the blood when they cannot be excreted by the kidney. Kidney failure in diabetes has

been linked to abnormalities of vasodilatation and could also be due to reactive oxygen species generated by endothelial derived nitric oxide (NO).[28] The result of the present study showed a marked elevation ($p<0.05$) in the urea and creatinine levels of the streptozotocin induced type 2 diabetic rats. Elevated urea and creatinine levels are a clear indication of kidney damage. Ameliorative effect was observed in the urea level of all the treated groups

Electrolytes are important for the normal functioning of the body. They are involved in many of the body processes including the maintenance and control of acid-base balance, control of the level of body fluid, they are important for blood clotting processes and they are also involved in nerve conduction.[29] Glucose homeostasis is a highly organised process, any defect in this process leads to increase in blood glucose levels (hyperglycemia).[30]Disturbances in water and electrolyte have been shown to be one of the complications arising from diabetes due to deficiencies in insulin secretion and high blood sugar. It has been noted that due to hyperglycemia, serum osmolality is increased leading to movement of water out of the cell hence reduction of serum sodium levels ([Na+]) by dilution.[31]

In the present study, treatment with the combined extract led to a decreased in serum sodium and potassium levels of the rats. Hypernatremia has been reported in human diabetic subjects.[32]This is attributed to Na+-K+-ATPase dysfunction and changes in intra-erythrocyte. Liamis et al., 2014, reported a high incidence of increased blood potassium levels, this the Authors attributed to redistribution of potassium from the intracellular to the extracellular compartment.[31] This is in agreement with the present studies, an increase was observed in the blood potassium concentration of the type 2 diabetic animal. In normal glucose homeostasis, in the presence of post prandial glucose a cascade of events lead to the closure of ATP-dependent potassium channels, resulting in cell depolarization, influx of calcium ions ($Ca^{2+}$) and eventual release of insulin.[33] Disturbances in glucose homeostasis distort this process hence release of potassium into the cells. Increase in plasma potassium levels in diabetic could also be due to reduced glomerular filtration of potassium $K^+$ resulting from acute kidney injury and chronic kidney disease.[31]

The result of this research also showed a marked increase in the serum $ca^{2+}$ and chloride levels of the type 2 diabetic rats. A high level of calcium in the serum of type 2 diabetics has been linked to hyperthyrodism due to a prolonged insulin resistance and insulin insufficiency.[31] Uncontrolled diabetes mellitus presents a lot of complication including hyperthyroidism hence the need to evaluate the level of calcium in the blood of diabetic patients. Research has shown that severe hypercalcemia or elevated blood calcium levels could also result from dehydration in metabolic acidosis. Hypercalcemia has also been linked to diabetic patients on some diuretics such as thiazide diueretics.[31]Hasona and Elasbali (2016) have demonstrated elevated calcium and chloride levels in type 2 diabetic subjects.[29]However, treatment of wistar albino rats with combined ethanol extract of A. muricata and A. heterophyllus resulted to a marked reduction in the calcium and chloride levels of the rats in group 4 and 5. Treatment with metformin had a better ameliorative effect on the calcium levels of the wistar albino rats after treatment for 30 days from 1.60±0.46 Mmol/l to 1.26±0.14 Mmol/l and 1.34±0.26 Mmol/l respectively while a better effect was observed in the chloride level of the rats treated with 100mg/kg of combined extract of A. muricata and A. heterophyllus.

The effect of combined ethanol extract ofA. muricata and A. heterophylluson the bicarbonate levels of the animals were also analysed, the result of this study showed an increased in the plasma bicarbonate level of diabetic control group. Treatment with 100 and 200mg/kg of combined ethanol extract of A. muricata and A. heterophyllus led to a reduced plasma bicarbonate level. The bicarbonate ion acts as a buffer to maintain the normal levels of pH (acidity or alkalinity) in the blood and other body fluids. It is important to measure the plasma bicarbonate level so as to ascertain the pH of the blood. Bicarbonate is secreted by the pancreas since it is the chief organ responsible for acid-base haemeostasis. Hence, any distortion in the normal functioning of the pancreas leads to defect in the secretion of bicarbonate. The result of this research showed reduced plasma bicarbonate after treatment with 100 and 200mg/kg body weight of combined ethanol extract of A. muricata and A. heterophyllus.

## V. Conclusion

This research shows that combined ethanol leaf extract of A. muricata and A. heterophyllus has ameliorative effect against streptozotocin induced type 2 diabetes mellitus. It also has a positive effect on liver and kidney parameters. This effect is due to the rich phytochemical content of the plant. This research also confirms the ethno pharmacological use of these plants in the treatment of diabetes mellitus.

**Conflicts of interest**

All authors declare no conflicts of interest.

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