IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 6, Issue 2 (Mar. – Apr. 2020), PP 39-46
www.iosrjournals.org

Effects of Ethanol Leaf Extract of Justicia Carnea on Biochemical Indices of Alloxan-Induced Diabetic Rats.

Ani, O.N[1], Udedi, S.C[2], Akpata, E.I[1], Ezeigwe, O.C[2], Oguazu, C.E[2],Onyishi, C.K[1], Nwakaudu, E.N[1].

[1]*Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria.*
[2]*Department of Applied Biochemistry, Faculty of Biosciences, NnamdiAzikiwe University, Awka, Anambra State, Nigeria.*

Abstract:
Background*: Justicia carnea is a medicinal plant reported to have diverse pharmacological functions including blood boosting potentials. Diabetes mellitus is one of the most common endocrine disorders accompanied with many metabolic syndromes. Use of herbal medicines has always been an option to treat a great number of diseases such as cancer, diabetes and its complications. The aim of this study was to evaluate the effects of ethanol extract of Justicia carnea on the biochemical parameters of alloxan-induced diabetic rats.*
Materials and Method*: Acute toxicity test was done using Lorke's method. Thirty (30) albino wistar rats of both sexes were assigned into five (5) groups of six (6) rats. All rats, except the normal control group, were induced with diabetes by single intraperitoneal injection of 150 mg/kg alloxan. Group A (normal control) received water, group B received the standard drug; glibenclamide (0.1mg/kg) orally, Group C (diabetic control) received water while Groups D and E received 100 and 200mg/kg body weight of the extract orally once per day respectively. Treatment lasted for 14 days. At the end of the treatment period, fasting blood glucose level was determined and blood samples were collected by cardiac puncture from the animals for the evaluation of the serum concentrations of biochemical parameters.*
Results: *There wasa significant ($p<0.05$) reduction in the fasting blood glucose levels of the animals treated with extract compared with the diabetic untreated rats. The Serum levels of AST, ALT and creatinine decreased significantly ($p<0.05$) while the ALP, urea and total protein levels increased in the diabetic rats treated with the leaf extracts compared with the untreated diabetic rats.Diabetictreated groups showed non-significant decrease ($p>0.05$) in serum levels of sodium, potassium, chloride and bicarbonate. There was a significant reduction ($p<0.05$) in the serum levels of HDL, LDL and TC with a non-significant increase ($p>0.05$) in the serum levels of TG and VLDL in diabetic rats treated with 100mg/kg extract. At 200mg/kg dose, the serum levels of LDL, VLDL, TG and TC were significantly reduced ($p<0.05$), while the serum level of HDL was increased non-significantly ($p>0.05$) when compared with the untreated diabetic control group.*
Conclusion*: The result shows that the Justicia carnea besides its hypoglycemic action will be useful in reducing the complications and metabolic syndrome which often coexist in diabetes.*
Key Word*: Alloxan-induced, Diabetic, Justicia carnea, ethanol, Biochemical parameters, Lipid profile*

Date of Submission: 27-03-2020 Date of Acceptance: 15-04-2020

I. Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia due to defects in insulin secretion and action or both[1]. It occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces[2]. Diabetes mellitus remains one of the age-long chronic diseases of the human race and its frontiers are expanding by the day. In diabetes, alteration of liver and kidney functions indices, lipid abnormalities, anaemia have been implicated as major risk factors to the progression of microvascular and macrovascularcomplications[3]. Besides hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis[4].

Liver is said to be the largest organ in the body with the gall bladder situated under it along with parts of the pancreas and intestines. These organs work together to digest, absorb, and process food. The liver's main job is to filter the blood. It also plays a role in detoxification of chemicals, drug metabolism and secretion of bile into the intestines. The regulation of insulin is mostly done by the liver. Insulin action in liver coordinately regulates lipid synthesis and glucose production by cell autonomous and non-autonomous mechanismsbut in type 2 diabetes mellitus (T2DM), insulin fails to suppress hepatic glucose production but promotes lipid

synthesis leading to hyperglycemia and hypertriglyceridemia[5]. Insulin affects many sites of mammalian lipid metabolism and stimulates synthesis of fatty acid in liver adipose tissue and in the intestine[6]. The elevated reactive oxygen species and the simultaneous decline in antioxidativedefence mechanisms observed in diabetic condition could promote the development of late complications[7]. To reduce the risk of late complications and other deleterious consequences of diabetes mellitus, such as blindness, renal failure, limb amputation, the control of blood glucose and lipid profile levels is necessary[8]. In diabetes disturbances in lipid profiles are seen, especially, an increased susceptibility to lipid peroxidation[9]. Furthermore, in the last stages of diabeteslipid metabolism is affected and seen as hyperlipidemia and hypercholesterolemia which are risk factors in atherosclerosis[10].

The kidneys play a vital role in the excretion of waste products and toxins such as urea, creatinine and uric acid, regulation of extracellular fluid volume, serum osmolality and electrolyte concentrations, as well as the production of hormones like erythropoietin and 1, 25dihydroxyvitamin D and renin[11]. The functional unit of the kidney is the nephron which consists of the glomerulus, proximal and distal tubules, and collecting duct. Assessment of renal function is important in the management of patients with kidney disease or pathologies affecting renal function[11]. Tests of renal function have utility in identifying the presence of renal disease, monitoring the response of kidneys to treatment, and determining the progression of renal disease[11]. It is a known fact that kidney function is compromised in uncontrolled diabetes mellitus[12]. Glycosuria, a cardinal and diagnostic feature of diabetes imposes dehydration via glucose osmotic diuresis. This dehydration is accompanied with severe loss of electrolytes including sodium, potassium, calcium, chloride and phosphates[12]. Also in diabetes there is abnormally increased ketone body formation leading to ketonuria. Ketone bodies being moderately strong acids, on excretion carried along side with them buffer cations particularly alkaline cations (Na+ and K+) and also bicarbonates[13]. Additionally, substances otherwise not present in urine are excreted in urine including albumin (microalbuminuria) in diabetic condition. This undue passage distorts the repellent ability of structural polysaccharides (e.g., hyaluronic acids) whose function is to maintain the integrity of the kidney cells[13], hence a distortion in the kidney basement membrane cell integrity. A combination of these factors and many more culminates in compromised kidney function in diabetes mellitus.

Medicinal plants have been documented as having beneficial properties used for the management of various ailments. Justicia carnea is a flowering plant that belongs to Acanthaceae family[14]. It is a medicinal plant used widely in Nigeria reported to have diverse functions, including blood- boosting potential.Most pregnant women and some sick individuals in the rural areas of Nigeria use the plant (called blood root) as a traditional blood supplement for the management of anemia[15]. Several species of Justicia are used traditionally in the management of various ailments such as inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhea, liver diseases, rheumatism and arthritis[16, 17].The plant has a number of secondary metabolites and phytochemicals such as flavonoids,alkaloids, essential oils, vitamins, fatty acids and salicylic acid [18, 19] that may be responsible for its observed therapeutic uses. Despite the use of Justicia carnea and other reported scientific studies, there are no reports on its effects on the major organs of the body. Therefore,it is imperative to investigate the effects of the plant on the essential biochemical indices in diabetic rats so as to establish its safety.

II. Materials and Methods

Plant Materials: The fresh leaves of Justicia carnea were used for this study.

Collection and Preparation of Plant Extract: Fresh matured leaves of Justicia carnea were harvested from farmlands in Awka, Anambra state. The fresh leaves were identified and authenticated by a taxonomist in the department of forestry, college of natural resources and environmental management, Michael Okpara University of Agriculture, Umudike, Abia state. The leaves of Justicia carnea were removed from the stems, sorted, rinsed and dried under shade. The dried leaves were ground into fine powder using an electric grinder and weighed using an electronic weighing balance after which 100g of the coarse powder was extracted in 80% ethanol by stirring intermittently, soaked for 72hours and filtered through Whatman no 4 filter paper. The filtrate was concentrated by heating in a water bath at 40ºc to produce crude ethanol extract of Justicia carnea.

Experimental Animals:Thirty (30) albino rats of both sexes weighing 100 – 150g were purchased from the animal house of the department of Veterinary Medicine, University of Nigeria Nsukka. They have been domesticated for purely scientific experiments. The animals were bred and kept in the animal house of the department of Applied Biochemistry of Enugu State University of Science and Technologyunder normallaboratory conditions (temperature and humidity) in a 12hours light and 12hours dark cycle. They were allowed to acclimatize for one week while being fed with standard laboratory animal pellets and water for two weeks.

Acute toxicity study: The LD_{50} of the extract was determined in rats using the method of Lorke[20]. Thirteen test animals were used. The study was in two phases. In the first phase, the animals were grouped into three with three animals each and given three different low doses of the extract; 10mg/kg, 100mg/kg and 1000mg/kg

respectively. They were watched for 48hrs for signs of toxicity and death. Thereafter, the second phase was conducted in which another set of animals were grouped into three groups of one animal each. They were given three different high doses of the extract; 1600mg/kg, 2900mg/kg and 5000mg/kg respectively.

Induction of Diabetes: Diabetes was induced by a single intra-peritoneal injection of alloxan monohydrate at a dosage of 150mg/kg using distilled water as vehicle. The alloxan injection was given to rats after the rats were fasted for 12hrs.The animals were given glucose solution to drink immediately after induction to reduce the drug induced hypoglycemia. Forty-eight (48) hours after alloxan administration, blood samples were drawn from tail and glucose levels determinedusing One Touch Glucometer and test strips to confirm diabetes. Rats with a fasting blood glucose concentration greater than 200mg/dl were considered diabetic and selected for the experiment.

Experimental Protocol: The 30 albino rats were divided into five (5) groups of six (6) rats each.

Group A – Normal control group, non-diabetic rats received food and water

Group B – Diabetic control received glibenclamide (5mg/kg)

Group C – Diabetic untreated group

Group D – Diabetic rats received 100mg/kg of ethanol extract of Justicia carnea

Group E – Diabetic rats received 200mg/kg of ethanol extract of Justicia carnea

Treatment was administered once a day by gastric intubation for 14days.

Sample Collection and Preparation: At the end of 14 days, the rats were fasted overnight and then anaesthetized with chloroform inhalation and sacrificed. Whole blood samples were obtained by cardiac puncture from each of the rats into well-labelled plain tubes and allowed to cloth for 2hours. The sample was then centrifuged at 4000rpm for 30minutes to recover the serum for the various biochemical assays.

Biochemical Assays

Assay for Kidney Function:The serum potassium content was determined using the method of Kabiru[21]. The concentration of potassium in the serum was read from Auto-analyzer using the potassium Cal as reference standard. The serum content of Sodium and Chloride were assayed using the method ofSkeggs and Hochestrasser[22]. The concentrations were read with Mindray Auto-chemistry Analyzer. Serum urea and creatinine level were determined using Randox reagent kit and the concentration of urea was determined with auto analyzer.Bicarbonate was determined using the method of Roger et al [23] using Agapee diagnostic kit. The bicarbonate content was read from Autochemistry Analyzer.

Assay for Liver Function: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined using Randox diagnostic kits according to the method of Limdi and Hyde[24].Total Protein was determined using Biuret method[25]and determined spectrophotometrically at 540nm.

Assay for Lipid Profile: Lipid profile was determined according to the method of Sidhu and Naugler[26] with slight modification.Cholesterol and Triglyceride content of the serum were measured at 546nm using Autochemistry Analyser (Mindray BA-88). LDL (low density lipoprotein) was determined by measuring the amount of cholesterol remaining in the serum after precipitation with polyvinyl sulphate. The LDL content is the difference between the total cholesterol and the cholesterol remaining in solution after precipitation. This was analyzed using Auto-chemistry Analyzer. VLDL (Very low density lipoprotein) was estimated by dividing triglyceride (TG) value by 5. HDL (High density lipoprotein) was determined by measuring the amount of cholesterol remaining in the serum after precipitation of LDL, VLDL and Chylomicron by the addition of phosphotungstic acid and magnesium chloride. The HDL content was measured as the remaining cholesterol in the sample solution after precipitation.

Statistical Analysis: Data analysis was performed using SPSS version 7 statistical package. Values were expressed as mean ± SD. Statistical significance of the results between groups was determined using one way analysis of variance (ANOVA). Differences between means were considered significant at $P<0.05$.

III. Result

Table 1 shows the blood glucose level determined before diabetes induction, 48 hours after induction and day 14 after treatment. Within 48hrs after induction of diabetes, there was increase in blood glucose level in all the rats as shown on day 2. After treatmentthere was a significant decrease in blood glucose level in diabetic treated groups (groups D and E)when compared with diabetic untreated group as shown on day 14.

Table 1: Fasting blood glucose level of the normal rats and the treated alloxan-induced diabetic rats.

Group	Initial glucose level before induction	48hrs after diabetes induction)	Day 14 (after treatment)
A (normal control)	66.00 ± 9.57	84.33 ± 5.24	106.16 ± 8.81
B (5mg/kg glibenclamide)	68.33± 17.04	> 600	94.33 ± 8.14
C (diabetic untreated)	78 ± 21.21	>600	301 ± 0.00
D (100mg/kg extract)	83.00 ±22.62	>600	204 ± 48.08
E (200mg/kg extract)	80.5 ± 7.78	>600	114 ± 35.35

Table 2 shows serum urea and creatinine concentration of the normal control, diabetic rats treated with Glibenclamide, untreated diabetic control, and diabetic rats treated with 100 and 200mg/kg of ethanol extract of Justicia carnea respectively. The ureaconcentrations were increased significantly (P<0.05) in treated groups in comparison with Groups A and C (normal and untreated diabetic control). However, treatment with Justicia carnea extract non-significantly decreased (P>0.05) the serum creatinine concentration similarly with the normal control while the creatinine concentration of group C (untreated diabetic control) was significantly increased (P<0.05) (0.35±0.00). Creatinine level of group D treated with 100mg/kg extract of Justicia carnea was 0.25±0.02mg/dl while that of group E treated with 200mg/kg extract of Justicia carnea was 0.23±0.05 as shown. Group C (untreated diabetic control) showed significant increase (p<0.05) in serum sodium (Na) concentration (130±0.00mmol/dl) in comparison with group A (normal control - 107±18.44mmol/dl). The administration of ethanol leaf extract of Justicia carnea in groups D and E significantly decreased (P<0.05) the sodium concentration similar to that of group A (normal control). In group D and E treated with 100 and 200mg/kg, the sodium concentrations were 122.00±15.56mmol/dl and 107.00±10.49mmol/dl respectively. The potassium concentration was reduced non-significantly (p>0.05) in the treated groups in comparison with the untreated diabetic group (C) and normal group (A). The chloride concentration reduced significantly (p<0.05) in group D treated with 100mg/kg of ethanol leaf extract of Justicia carnea but increased non-significantly in other groups in comparison with the normal control. The bicarbonate concentration was reduced non-significantly (p>0.05) in group E treated with 200mg/kg of ethanol extract of Justicia carnea but was increased non-significantly (p>0.05) in other groups when compared with the normal control. There was also a significant increase (P<0.05) in the bicarbonate level of the diabetic untreated group (C).

Table 2: Effects of Justicia carnea leaf extracts on the kidney function parameters in alloxan-induced diabetic rats in comparison with the control.

Group	Urea (mg/dl)	Creatinine (mg/dl)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	$HCO3^-$ (mmol/L)
A(normal control)	$7.50±4.05^b$	$0.28±0.01^a$	$107.00 ±18.44^a$	$4.25±1.22^a$	$85.17±15.01^b$	$19.5±3.56^a$
B(5mg/kg glibencla - mide	$9.13±1.18^a$	$0.26±0.01^a$	$129.40 ±12.84^b$	$3.40±1.30^a$	$94.80±24.71^b$	$23.8±6.98^a$
C(diabetic untreated)	$8.71±0.00^b$	$0.35±0.00^b$	$130.00±00^b$	$4.20±0.00^a$	$98.00±0.00^b$	$25.00±0.00^b$
D(100mg/kg extract)	$11.85±0.00^a$	$0.25±0.02^a$	$122.00±15.56^b$	$3.17±1.32^a$	$55.50±54.45^a$	$20.00±0.00^a$
E(200mg/kg extract)	$12.94±2.35^a$	$0.23±0.05^a$	$107.00±10.49^a$	$2.25±0.53^a$	$91.00±26.09^b$	$18.00±4.16^a$

Values represent mean ± SD. Means with the same letterare not significantly different from each other (P>0.05).

Table 3 shows the effects of administration of Justicia carnea leaf extract on the liver enzymes and Total protein in alloxan-induced diabetic rat in comparison with the control.There was significant increase (p<0.05) in the AST (aspartate transaminase) and ALT (Alanine transaminase) of the diabetic untreated group (group C). The administration of ethanol leaf extract of Justicia carnea at 100mg/kg and 200mg/kg in groups D and E respectively causedsignificant reduction (P< 0.05) similarly to the normal control (A) and the diabetic group (B) treated with the standard drug; glibenclamide. The maximum reduction in ALT and AST was observed at 100mg/kg dose and 200mg/kg respectively. The 200mg/kg treated group was comparable with the normal control with no significant difference. Furthermore, ALP (Alkaline Phosphatase) was significantly reduced (p<0.05) in the diabetic untreated group and significantly increased (p<0.05) in the extract treated groups similar to the normal control group and diabetic group treated with the standard drug. The induction of diabetes in rats non-significantly increased (P<0.05) the level of total protein in diabetic untreated group when compared with the normal control group. Treatment of diabetic rats with the extract as well as with the standard drugs non-significantly increased (p<0.05) the total protein.

Table 3: Effects of ethanol leaf extract of Justicia carnea on liver function indices and protein level in diabetic rats in comparison with the control.

Group	AST(U/L)	ALT(U/L)	ALP(U/L)	Total Protein (mg/ml)
A(normal control)	$53.30±9.59^b$	$16.88±5.56^b$	$27.30±4.12^b$	$1.86±0.05^a$
B (5mg/kg glibenclami-de	$53.24±10.87^b$	$12.94±0.93^b$	$28.50±2.86^b$	$3.12±0.10^a$
C(diabetic untreated)	$73.25±0.00^a$	$31.75±0.00^a$	$19.61±1.11^a$	$2.24±0.00^a$
D(100mg/kg extract)	$49.58±16.42^b$	$7.06±3.40^b$	$31.12±5.21^b$	$4.46±1.15^a$
E(200mg/kg extract)	$30.82±13.32^b$	$18.42±6.00^b$	$42.86±2.11^b$	$3.96±0.90^a$

Values represent mean ± SD. Means with the same letter are not significantly different from each other (P>0.05).

Table 4 shows the effects of ethanol leaf extract of Justicia carnea on the lipid profile of the diabetic rats in comparison with the control. The level of total cholesterol (TC) was significantly higher ($p<0.05$) in untreated diabetic control group (C) than in the normal control group (group A). All doses of the extract significantly decreased ($p<0.05$) the serum level of total cholesterol with maximum reduction observed in group D treated with 100mg/kg. There was no significant difference ($P<0.05$) between the extract dose of 200mg/kg and group B treated with the standard drug. There was a significant decrease ($p<0.05$) in the serum triglyceride (TG) in the group treated with 200mg/kg of the extract but a non-significant increase ($p<0.05$) was obtained in the group treated with 100mg/kg of the extract when compared to untreated diabetic control group (C). In the glibenclamide treated group and 200mg/kg extract treated group there was no statistically significant difference ($p>0.05$) when compared to the normal group (A). All doses of the extract non-significantly increased the serum High density Lipoprotein (HDL) with maximum increase observed in group E treated with 200mg/kg when compared with the normal group (A). There was no significant difference ($p>0.05$) between the extract dose of 200mg/kg and diabetic control (Control C). There was a significant decrease ($p<0.05$) in the serum Low density Lipoprotein (LDL) in groups administered with 200mg extract and glibenclamide, with marked decrease recorded in the groups treated with 200mg/kg extract (group E). There was a non-significant increase in the serum LDL in untreated diabetic group when compared with the treated groups. The effect of 200mg/kg extract on the serum Very low density Lipoprotein (VLDL) level was similar to that of the normal group (group A). The serum VLDL level was significantly increased ($p<0.05$) in all other doses and the untreated diabetic group with maximum increase in group D treated with 100mg/kg extract.

Table4: Effects of ethanol leaf extract of Justicia carnea on lipid profile in diabetic rats in comparison with the control.

Group	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
A (normal control)	16.45±1.28[a]	18.60±1.07[a]	3.36±0.54[a]	6.25±1.05[b]	3.29±0.26[a]
B (5mg/kg glibencl-amide	21.86±0.97[a]	10.98±1.44[a]	4.03±0.96[a]	2.97±0.79[a]	4.37±0.19[b]
C (diabetic untreated)	45.32±00[b]	34.33±0.00[b]	7.26±1.02[a]	4.41±1.31[a]	9.06±00[b]
D (100mg/kg extract)	56.25±1.15[b]	7.48±2.26[a]	6.86±1.01[a]	3.58±1.01[a]	11.25±0.23[b]
E (200mg/kg extract)	17.28±4.12[a]	12.64±1.48[a]	7.81±0.99[a]	1.69±0.54[a]	3.45±0.82[a]

Values represent mean ± SD. Means with the same letter are not significantly different from each other ($P>0.05$).

IV. Discussion

From the result of the acute toxicity (LD50) test, the extract had an acute oral LD50 greater than 5000mg/kg in rats. This shows high margin of safety[20]. The result from the fasting blood glucose test showed a reduction in the blood glucose levelof the rats following the administration of the leaf extract and this justifies its usage in folkloric medicine in the treatment of diabetes. The pancreatic β-cell is destroyed through alloxan-mediated oxidative damage inducing diabetesby depriving the animal of insulin[27]. Justiciacarnea's ability to correct and reverse diabetes induced by alloxan can as well correct complications arising from the β-cell destruction. In this study it was observed that induction of diabetes caused some pathological changes in the animals. The significant increase observed in the serum urea and creatinine of the diabetic groups as against the normal control group could be attributed to increased synthesis from the damaged pancreatic cells caused by alloxan injection. This is so because deficiency of hormone and consequent inability of glucose to get to the extra-hepatic tissues stimulates gluconeogenesis as an alternative route of glucose supply[28]. This process is sustained by increased release of free glucogenic amino acids circulated in plasma which are deaminated in the liver leading to increased urea in the blood[29]. Urea is synthesized in the liver from ammonia during deamination of proteins and thus it is the breakdown product of amino acid and protein metabolism. Excess nitrogen is removed from the body through filtration of urea from the blood into the urine by the renal glomeruli[30]. Serum urea level also increased significantly ($p<0.05$) in the group of diabetic animals administered the leaf extracts when compared with the normal control (Table 2). This increase might therefore arise from the damaged pancreatic cells caused by alloxan which was not reversed by the extract administration. Creatinine is a metabolite of muscle creatine and the amount in serum is said to be proportional to the body's muscle mass [29] Amount of creatinine is always constantand so increasedlevels indicate reduced renal function since it is easily excreted by the kidneys [31]. Elevation of creatinine levels shows that more creatinine was retained in the blood. Diabetes increased the level of creatinine as evidenced in the untreated diabetic group (C). However, following administration of the extracts the creatinine level was significantly reduced ($p<0.05$) as observed in groups D and E. The effect of the extract was dose dependent, higher dose of the leaf extracts produced more effect (reduced creatinine retention). This indicates a protection against renal impairment arising from diabetes complications by the leaf extracts.

In the diabetic, electrolyte imbalance is a common feature arising from altereddistribution of electrolytes[32]. It is also related to hyperglycemia- induced osmotic fluidshifts or of total-body deficits brought about by osmoticdiuresis[33]. Electrolyte imbalance frequently occurs due to kidney failure, dehydration, fever and vomiting, thereby disturbing normal cellular functions[34]. Electrolytes balance in the blood is a good indicator of how well the kidneys and heart are functioning[34]. Diabetes mellitus produces dysnatremias through several underlying mechanisms[35]. Glucose is one of the osmotically active substance and its excretion in urine by diabetes imposes an osmotic diuresis[30] with the consequences of electrolyte lost with dehydration. In hyperglycemia this will increase osmolality of the serum, which results in movement of water out of the cells. Uncontrolled hyperglycemia also induceshypovolumichyponatremia due to osmotic diuresis. From this study there were alterations caused by diabetes in the serum electrolytes of the animals as observed in the untreated diabetic group. The serum electrolytes levels of the untreated diabetic groups were observed to increase when compared with the normal control group. Upon administration of the leaf extracts, there was restoration of the altered electrolytes which is an indication that the extract protected the kidneys from impairment caused by diabetes.

Enzyme activities in the tissues are often used as 'marker' to ascertain early toxic effects of administered foreign compounds to experimental animals [35, 36]. This is because, any alterations in the biochemical processes in experimental animals due topresence of a xenobiotic would reflect as increase ordecrease in the activity of such enzymes[37, 38]. AST, ALT and ALP are marker enzymes which are indicators of liver injury or impairment in liver functions. They play important roles in metabolism [39]. ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes. AST catalyses the reductive transfer of amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. ALT plays an important role in gluconeogenesis and amino acid metabolism. It catalyses the reductive transfer of an amino group from alanine to α-ketoglutarate to yield glutamate and pyruvate. In liver damaged with hepatocellular lesions, these marker enzymes are released from damaged tissues and their levels increase in blood flow[40]. ALT and AST are two well-known diagnostic indication of liver damage. The ratio of these two enzymes in the serum is used to differentiate liver damage from other organ damage [41]. Infact, ALT activity is the most frequently relied biomarker of hepatotoxicity. Hepatotoxicity leads to elevation of the normal values of these enzymes due to the body's inability to secrete them through bile from congestion or obstruction of the biliary tract that may occur within the liver. Elevated serum activity of this enzyme is usually observed during liver damage. In this study, diabetes induced damages to the liver. These damages resulted in an increase in serumtransaminases as observed in untreated diabetic control group (C).There was a significant increase in ASTand ALT levels and decrease in ALP. Administration of the ethanol extract of Justicia carnea at 100mg/kg and 200mg/kg significantly ($P<0.05$) decreased the disturbed AST and ALT values and increased the ALP value. These effects by the extracts are comparable with that of glibenclamide (the standard drug) and the normal control group (B).This ability of the ethanol extract of Justicia carnea to lower these values may suggest hepatoprotective activity of Justicia carnea leaves. This activity would be manifested by stabilization of the hepatic membrane and regeneration of the hepatocytes as suggested by Effoet al[42]. The extract may also have prevented the release of hepatic enzymes at the level of the blood stream by a reduction of the tissue lesions [43]. The increase in ALP value following administration of the extract when compared to the untreated diabetic control group (C) may have been due to disturbances in the secretary activity or in the transport of metabolites or other hepatotoxic conditions as suggested by Sharma and Shukla [44]. More so, at the highest dose level of 200mg/kg administered, the maximum increase observed could also be drug induced as observed with over-dose of paracetamol, methyl dopa, isonocizid and certain steroids[45]. Alkaline phospatase are useful in diagnosis of hepatobilliary or cholesterol obstruction[46]. A decreased level of total protein was also observed in untreated diabetic control group when compared to the extract treated groups (D and E). The administration of the extract caused an increase in total protein when compared to the other groups which indicates stabilization of plasma membrane and protection of liver cell membrane. It also implies that the liver's synthetic activity and ability to maintain nutrient homeostasis was enhanced as a result of the administration of the Justicia carnea extract.

Increase in the level of lipids such as total cholesterol and triglyceride is one of the metabolic alterations arising from diabetes[47].It has been reported that high level of cholesterol, particularly total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL) are mainly responsible for the onset of coronary heart diseases[48]. In the present study, diabetes caused an increase in the lipid profile of the rats. Administration of 100mg/kg ethanol leaf extract of Justicia carnea reduced serum level of high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol (TC), but increased the serum level of triglycerides (TG) and very low-density lipoprotein (VLDL). However, at 200mg/kg dose, the serum levels of LDL, VLDL, TG and TC were reduced while the serum levels of HDL was increased. The effectiveness of the extract is thus observed to be dose-dependent. This hypolipidemic activities shown by the leaf extract might be due to ability of the extract to cause regeneration of the β-cells of the pancreas and potentiation of insulin secretion from surviving β-cells. It is also assumed to be mediated by a control of lipid metabolism by some of the phytochemicals present in the plant[49]. It has been reported that many nutritional factors such as saponins and tanniscontribute to

the ability of herbs to improve dyslipidemia[50]. Preliminary phytochemical screening of the extract revealed the presence of saponin, alkaloids, flavonoids, phenol[18]. This may be responsible for the lipid lowering effect observed. Saponins are known antinutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its fecal excretion[48]. The reduced cholesterol level by the extract may have contributed to the observed high serum HDL at 200mg/kg dose in the animals. About 30% of blood cholesterol is carried in the form of HDL-C. Significant lowering of total cholesterol and rise in HDL-C is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions [49]. HDL-C function to remove cholesterol antheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease[52]. Therefore, the observed increase in the serum HDL level on administration of 200mg/kg extract dose in alloxan-induced diabetic rats, indicates that the extract have HDL-C boosting effect. Furthermore, the stabilization of serum TG and TC levels in rats by the plants extract may be attributed to glucose utilization and hence depressed mobilization of fat[53]. Thus the plant extract may be useful in handling hyperlipidemia and hypercholesterolemia complications which often coexist in diabetes[54].

V. Conclusion

From this study it has been shown that the ethanol leaf extract of Justicia carnea has the potential to protect the vital organs from complications due to diabetes. This it does by reversing alterations and stabilizing the biochemical parameters which are indicators of organ failure and malfunctions in diabetes.

References

[1]. Kangralkar VA, Shivrajand DP, Bandivadekar RM. 0xidative stress and Diabetes: *A review. Inter. J. Pharmaceutical Applications*. 2010;1(1):38-45.
[2]. Krishnasamy S, Abell TL. Gastroparesis: Principles and Current Trends in Management. Diabetes Ther. 2018; 9(1): 1-42
[3]. Mandade R, Sreenivas SA. Antidiabetic effects of aqueous ethanolic extract of Hibiscus rosasinensis leave. On Streptozotocin-induced diabetic rats and possible morphologic changes in the liver and kidney. International Journal of phamacologym2007; 7: 363-369.
[4]. Saikat D, Sekhar KB, Ranabir S, Subhash CM. Antidiabetic effect of maturedfruits of Diospyros peregrine in Alloxan-induced diabetic rats. Int. J. Green Pharmacy. 2008; 2(2): 902-929.
[5]. Titchenell PM, Lazar MA, Birnbaum MJ. Unravelling the regulation of Hepatic Metabolism by Insulin. Trends EndocrinoloMetab. 2017; 28(7):497-505.
[6]. Suryawanshi NP, Bhutey AK, Nagdeote AN, Jadhavand AA, Manoorkar GS. Study of lipid peroxide and lipid profile in diabetes mellitus. Indian J. Clinic. Biochem. 2006; 1: 126- 130.
[7]. Brown DJ, Goodman J. A review of vitamin A, C and E and their relationship to cardiovascular disease. Clin. Excellence Nurs. Pract. 1998; 2: 10-12
[8]. Subbiah R., Kasiappan R., Karurannand S, Sorimuthu S. Beneficial effects of Aloe vera leaf gel extract on lipid profile status in rats with streptozotozin induced diabetes. Clin Experimental Pharmacol. Physiol. 2006; 33: 232-237.
[9]. Li X.M. Protective effective of Lyciumbarbarum polysaccharides on Streptozotocin induced oxidative stress in rats. Intl. J. Biol. Macromolecules. 2007; 40: 461-465.
[10]. Schwartz SL. Diabetes and dyslipidemias. Diabetes Obes. Metab. 2006; 8: 355-364.
[11]. Gounden V, Jialal I. 2019. Renal function tests. In: stat pearls. Treasure Island (fc): stat pearls publishing.
[12]. Gaw A, Cowmaru RA, O'Reilly DS, Shepherd J. Clinical Biochemistry,1995; New York: Churchill Livingstone
[13]. Ramsey L. A Synopsis of Endorcrinology and metabolism. 1986; 3rd Edn., Bristol: Wright
[14]. Corea GM. Chemical consistent and biological activities or species of Justicia: A review. Brazitiaphamarcognosis 2012; 22:220-238.
[15]. Igbinaduwa PO, Kabari KM, Chikwue TC. Phytochemical and Anti-Anaemic Properties of Ethanol Leaf Extract of JusticiacarneaVahl (Acantheceae). Nigerian Journal of Pharmaceutical and Applied Science Research. 2019; 8(2): 55-61.
[16]. Badami S, Aneesh R, Sankar S, Sathishkumar, MN, Suresh B. Antifertility activity of Derris brevipes variety coriacea. Journal of Ethnopharmacology. 2003; 84: 99-104.
[17]. Corrêa GM, Alcântara AFC. Chemical constituents and biological activities of species of Justicia - a review. Brazilian Journal of Pharmacognosy. 2012; 22(1): 220-238.
[18]. Orjiakor CA. Studies on the phytochemical and nutritional composition of Aqueous leaf extract of Justicia carnea and its effect on some Biochemical parameters in anaemic rats. 2012; M.Sc. research project in nutritional Biochemistry; department of Biochemistry, University of Nigeria, Nsukka.
[19]. Al-Juaid SS, Abdel-Mojib MA. A novel podophyllotoxin lignin from Justiciaheterocarpa. Chemical and Pharmaceutical Bulletin. 2004; 52: 507-509.
[20]. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983; 54:275-287.
[21]. Kabiru AY, Tahir V, Garba MH, Kuta FA, Jibril A, Shuaib MA. Changes in Serum Electrolyte Levels in Typhoid Fever Patients Attending Minna General Hospital, Nigeria. British Journal of Medicine & Medical Research. 2016; 14(5): 1-7.
[22]. Skeggs LT, Hochestrasser HC. Colorimetric method of chloride estimation. Clinical Chemistry. 1964; 10:918–920.
[23]. Roger LF, Lester JW, Donald AS, Kenneth JP. Enzymatic Method for Determination of CO_2 in Serum. Clinical Chemistry. 1976; 22(2):243-245.
[24]. Limdi JK, Hyde GM. Evaluation of abnormal liver function tests. Postgraduate medical Journal. 2003; 79: 307-312.
[25]. Sidhu D, Naugler C. Fasting Time and Lipid Levels in a Community-Based Population. Archives of Internal Medicine. 2012; 172 (22): 1–4.
[26]. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 1949; 177:751-66.
[27]. Szkudelski T. The mechanism of alloxan and streptozotocin action in $ cells of the rat pancreas Physiol. Res. 2001; 50: 537-546.

[28]. Robinson G, Johnston DE. Metabolic disorder: Diabetes. In: Mechanisms of Disease. An Introduction to Clinical Science. 1997; IstEdn., Cambridge: Cambridge University Press.
[29]. Atangwho IJ, Ebong PE, Egbung GE, Eteng MU, Eyong EU. Effect of Vernoniaamygdalina Del. on liver function in alloxan-induced hyper glycemic rats. J. Pharm. Biores. 2007c; 4(1): 1- 7.
[30]. Nwodo OFC, Joshua PE, Ugwuoke MC, Uroko RI. Anti-malaria and some biochemical indices of the ethanol extract of Zapotecaportoricensis root on malaria infected mice. Asian Journal Biochemistry. 2015; 10(6): 281-289.
[31]. Loeb S. Clinical Laboratory Test: Values and Implication. Copyright, Springhouse Corporation, Springhouse, Pennsylvania. 1991
[32]. SarguruDatchinamoorthi, Vanaja R, BalajiRajagopalan Evaluation of Serum Electrolytes in Type II Diabetes Mellitus. Int. J. Pharm. Sci. Rev. Res. 2016; 40(1): 251-253.
[33]. Palmer BF, Clegg DJ. Electrolyte and Acid–Base Disturbances in Patients with Diabetes Mellitus. The New England journal of Medicine. 2015; 373; 6.
[34]. Chernecky CC, Berger BJ. Electrolytes panel- Eds. Laboratory tests and diagnostic procedures, 6th ed. St Louis, Mo: Elsevier Saunders. 2013; 464-467.
[35]. Akanji MA, Ngaha EO. Effect of repeated administration of Berenil on urinary enzyme excretion with corresponding tissue pattern in rats. PharmacolToxicol. 1989; 64:272-279.
[36]. Adesokan AA, Akanji MA. Effect of administration of aqueous extract of Enantiachlorantha on the activities of some enzymes in the small intestine of rats. Nig J BiochemMol Biol. 2004; 18:103-105.
[37]. Kandem L, Siest G and Magdalou J. Differential toxicity of aflatoxin B1 in male and female rats, relationship with hepatic drug metabolizing enzymes. Biochem. Pharmacol. 1982; 31: 3057-3062.
[38]. Zilva JP, Pannal PR.1984. Liver disease and gallstone. In: Clinical chemistry in diagnosis and treatment. 1984; 313-335. Lloyd-Duke medical books, London,
[39]. Reitman S, Frankel S. A colorimetric determination of oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol. 1957; 28: 56-63.
[40]. Nkosi CZ, Opoku AR, Terblanche SE. Effect of Pumpkin seed (CucurbitaPepo) Protien Isolate on the Activity Levels of Certain Plasma Enzymes in CCL4-Induced Liver injury in low protein fed rats. Phyptherapy research. 2005; 19,341-345.
[41]. Nathwani RA, Pais S, Reynolds TB, et al. Serum alanine aminotransferase in skeletal muscle diseases. Hepatol. 2005; 41:380–2.
[42]. Effo KE, Kovakuo LS, Irie- Nguessan G, Kovakuo-Sirancy G. Antioxidant Activity and Hepatoprotective Effect of an Aqueous Extract of Alchorneacordifolice Leaves. Pharmacology and pharmacy. 2007; 8:369-380
[43]. Singh K, Singh N, Chandy A, Ganga Rao B. In vivo Antioxidant and Hepatoprotective activity of methanolic extracts of daucuscarota seeds in experimental animals. Asian pacific journal of Tropical Biomedicine. 2012; 2:385-388.
[44]. Sharma ARM, Shukla S. Hepatoprotective action of a proprietary herbal preparation against carbon tetrachloride intoxication. Indian drugs. 1995; 32:120-124.
[45]. Gray CH, Howorth PJN. Clinical chemical pathology Edward Arnild publishers. 1980: 80-99.
[46]. Johnson DF, Fody EP. Clinical chemical. Principles, procedure and corrections. 2nd edition. Philadelphia. J.B.Lipncotts Company. 1992: 473-478.
[47]. Vivek KS, Suresh K, Hitesh JP, Shivakkumar H. Hypoglycemic activity of Ficusglomerata in alloxan-induced diabetic rats. Int. J. Pharmaceutical Sci. Rev. Res. 2010; 1(2).
[48]. Jahnavi N, Naidu PP, Rao K, Karunasree CP. Antihyperlipidemic effect of Asparagus gonnoclados baker against cholesterol diet induced hyperlipidemia in rats. Asian J Pharm Clin Res 2014; 7(2):212-5.
[49]. Eze ED, Mohammed A, Musa KY, Tanko Y, Isa AS. Effect of Ethanolic Leaf Extract of Mucunapruriens (fabaceae) on Lipid Profile in Alloxan-Induced Diabetic Wistar rats. British Journal of Pharmacology and Toxicology. 2012; 3(3): 102-109.
[50]. Nimenibo-uadia, R. (2003). Effect of aqueous extract of canavaliaensiformis seeds on hyperlipedemic and hyperketonaemia in alloxan-induced diabetic rats. Biokemistri. 15, 7-15.
[51]. Schwenke EC, Carew TE. Initiation of atherosderotic lesions in cholesterol fed rabbits. Atherosclerosis. 1989; 9:895-907.
[52]. Kwiterovich PO. The metabolic pathways of high-density lipoprotein, low density lipoprotein and triglycerides: A current review. American J. Cardiology. 2000; 86(12): 5-10.
[53]. Iweala EEJ, Oludare FD. Hypoglycemic effect, biochemical and histological changes of spondiasmombin Linn and parinaripolyandrabenth. Seed ethanolic extracts in alloxan-induced diabetic rats. Journal of pharmacology and Toxicology. 2011; 6:101-112.
[54]. Sharma SB. Hypoglycemic and hypolipidemic effect of ethanolic extract of seed of Eugenia jambolana in alloxan diabetic rabbits. Journal of Ethnopharmacology. 2003; 85: 201-206.

Ani, O.N,etal. "Effects of Ethanol Leaf Extract of Justicia Carnea on Biochemical Indices of Alloxan-Induced Diabetic Rats." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB), 6(*2), (2020): pp. 39-46.