



## **Antidiabetic and Modulatory Effect of Ethanol Extract of Neem Leaf on Some Essential Biochemical Parameters of Streptozotocin-induced Diabetic Rats**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Author EFC designed the study. Author EOC performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/IJBCRR/2019/v28i430157

Editor(s):

(1) Dr. Halit Demir, Professor, Department of Chemistry, Faculty of Art and Science, Yil University, Yuzuncu, Turkey.

Reviewers:

(1) Sandra Machado Lira, Centro Universitário Maurício de Nassau, Brasil.

(2) Mehmet Evren Okur, University of Health Sciences, Turkey.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/54252>

**Received 15 November 2019**

**Accepted 20 January 2020**

**Published 25 January 2020**

**Original Research Article**

### **ABSTRACT**

The study was conducted to investigate the modulatory activity of ethanol extract of neem leaf on the essential biochemical parameters of streptozotocin-induced diabetic rats. The 24-hour acute toxicity test of the orally administered ethanol extract was determined using Finney's method. Diabetes was induced in the rats by a single intraperitoneal dose of 50 mg/kg<sub>bw</sub> of streptozotocin (STZ). Blood was collected after 28 days of treatment and used for biochemical analysis. Biochemical analysis was done using standard biochemical methods. The acute toxicity study of the ethanol extract reveal that the median lethal dose was 5.0 g/kg body weight. The anti-diabetic study carried out for a period of 28 days showed a significant ( $p<0.05$ ) reduction in the fasting blood glucose levels of the animals treated with ethanol extract compared with the diabetic untreated rats. The urea, creatinine, ALT, AST, ALP and bilirubin levels increased significantly ( $p<0.05$ ) in the diabetic untreated rats compared with the groups treated with the ethanol extract of neem leaf. The serum alpha-amylase activity of the treatment groups decreased significantly ( $p<0.05$ ) compared

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with the diabetic untreated group. The diabetic untreated rats showed a significant ( $p < 0.05$ ) decrease in their insulin level compared with the insulin levels of animals treated with the ethanol extract of neem leaf. The ethanol extract of neem leaf is efficacious in lowering blood glucose level in STZ-induced diabetic rats by regenerating the beta cells integrity which was seen from the increase in the insulin level of the extract treated rats. It modulates the essential biochemical parameters of diabetic rats favourably towards recovery and improved health.

**Keywords:** *Neem; leaf; ethanol; extract; biochemical; streptozotocin.*

## 1. INTRODUCTION

Diabetes is a disorder characterized by impaired metabolism of carbohydrates, proteins and fats due to inadequate or inefficient activity of insulin [1]. Diabetes mellitus is a chronic condition in which a person has a high blood glucose level, either because the body does not produce enough insulin, or because body cells do not efficiently respond to the insulin that is produced. Diabetes mellitus constitute a great public health problem globally [2]. It is the commonest endocrine-metabolic disorder in Nigeria paralleling the experience in other parts of the world [3].

Diabetes mellitus commonly known as hyperglycemia can result from the body's failure to produce insulin and is referred to as Type I diabetes mellitus. The majority of Type I diabetes is immune-mediated in nature, where beta cells loss can be a T-cell mediated autoimmune attack. Type I diabetes is caused by a lack of insulin or insufficient insulin due to the destruction of insulin-producing beta cells in the pancreas [4]. Diabetes can also be caused by insulin resistance (reduced sensitivity of cells to insulin), a relative insulin deficiency, or both. When this is the cause, it is called Type II diabetes and is usually noticed in adulthood, and occurs mostly in patients that are obese [1,5]. Diabetes mellitus may also be characterized by glucose intolerance during pregnancy which is associated with a variety of adverse birth outcomes, including excessive fetal weight gain and related increases in the rate of cesarean delivery and perinatal injury [6]. In this case, it is called Gestational diabetes mellitus. Gestational diabetes mellitus usually results after delivery [7].

Symptoms of diabetes mellitus include polyuria (frequent urination), polydipsia (increased thirst), weight loss, sometimes with polyphagia (excessive hunger) and blurred vision. Symptoms of type II diabetes may develop gradually and can be subtle; some people with type II diabetes remain undiagnosed for years

[8]. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia [9]. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome [10].

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction [11,12]. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease [9]. Patients with diabetes mellitus are more likely to develop and die from microvascular complications than the non-diabetic population [13]. Loss of hearing, eyesight, and cognitive ability has also been linked to this condition [14].

The economic burden of diabetes is enormous in terms of the direct cost of intensive monitoring and control of blood glucose and managing cardiovascular, renal, and neurological consequences [3]. This has resulted to psychological trauma in patients suffering from diabetes. In recent years, there has been renewed interest in plant medicine and plant-based therapies [15]. Many traditional plant treatments for diabetes mellitus are used throughout the world and some of these plants have been validated while a good number of them are yet to receive scientific scrutiny [16]. The use of medicinal plants to prevent and treat diabetes mellitus successfully over the years has attracted the attention of scientists globally. In the rural communities, many people depend solely on medicinal plants for the treatment of diabetes due to its easy affordability and availability even when the efficacy of the herbal remedy has not been established. *Azadirachta indica* Juss, Meliaceae (neem) is one of the most versatile medicinal plants with a wide spectrum of biological activity. Traditional healers make

use of the leaves of neem to treat diabetes. Scientific report supports the hypoglycemic activity of neem leaves [17]. While folklore medicine and few scientific studies suggest that it has hypoglycemic effect there are no reports as to how it modulates essential biochemical parameters in diabetes. This is the aim of this study.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection/Identification

The leaves of neem were collected from Nnamdi Azikiwe University, Awka. Anambra State. The sample was validated by a botanist in the Department of Botany, Nnamdi Azikiwe University, Awka.

### 2.2 Sample Preparation

The leaves were properly washed and air dried at room temperature for two weeks. The dried leaves were ground into powder using corona manual grinding machine. Exactly 100 g of the ground leaves of neem were soaked in 1 litre of ethanol for 24 hrs. It was sieved and filtered using Whatman no1 (125 mm) filter paper. The filtrate was evaporated to dryness using rotary evaporator and the paste was put in a stoppered universal bottle and stored in the refrigerator until needed. The paste was dissolved in distilled water before use.

### 2.3 Chemicals

Streptozotocin, manufactured by Sigma, Germany. All other chemicals used in this study were of analytical grade.

### 2.4 Experimental Animals

A total of one hundred (100) male rats were purchased from the animal house of Chris Farms, Awka and used for the study. They were maintained and housed in aluminium cages in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka. They were allowed to acclimatize with the environment for one week before use. The animals were fed water and guinea growers mash pellets obtained from Vital feed distributors, Awka *ad libitum*.

#### 2.4.1 Acute toxicity test (LD<sub>50</sub> determination) of neem leaf

The Median Lethal Dose (LD<sub>50</sub>) was determined using Wistar Albino rats. Test animals were

randomly divided into seven (7) groups of ten (10) rats each and administered graded doses of 0.5, 1, 2, 3.5, 4.5, 5 and 5.5 g/kg body weight. The neem extracts were administered by oral gavage using an intubation cannula and were monitored for 24 hours for changes in behaviour and mortality. The animals were monitored closely for signs of toxicity. The LD<sub>50</sub> were determined by plotting a graph of Probit against Log Dose according to Finney's method.

#### 2.4.2 Determination of anti-diabetic effect of ethanol extract of neem

A total of thirty (30) male wistar albino rats were used for the study. Twenty-five (25) of the rats were made diabetic and subsequently divided into five groups of five rats each. The remaining 5 non-diabetic rats were used as control subjects. Groups A, B and C were orally administered 100mg, 200 mg and 400 mg/kg body weight of ethanol extract of neem leaf respectively. Group D received 100mg/kg body weight of metformin (a standard drug used in the treatment of diabetes), group E did not receive any treatment and group F was a control group of 5 non-diabetic rats that received 1ml of distilled water in place of treatment regimen. The blood glucose levels of the rats were checked before the administration of Streptozotocin to 25 rats using One Touch Glucometer and test strips. The rats were then fasted for 16 hours, but with free access to water after which they received an intraperitoneal injection of streptozotocin 50 mg/kg body weight [18]. The rats were orally given 5ml each of 5% glucose solution 2 hours after administering streptozotocin to prevent hypoglycemia. The animals were allowed free access to food and water after streptozotocin injection. After 48 hours of the streptozotocin administration, blood was collected *orbital rectally* and the glucose concentrations were determined using a One Touch Glucometer (Life Scan, USA) and test strips based on the method of Trinder [19]. Diabetes was confirmed to have been induced when the fasting glucose level was observed to be far much higher than normal (between 60 mg/dl to 120 mg/dl) to above 200 mg/dl. Treatment was done for 28 days. While the treatment lasted, blood glucose levels were determined every two days (48 hrs) using One Touch Glucometer and test strips. Kidney and liver function tests as well as alpha amylase activity and insulin level were determined at the end of 28 days.

## 2.5 Kidney Function Test

Kidney function parameters  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$  and  $\text{HCO}_3^-$  were measured using routine diagnostic techniques by autoanalyser, Selectra Junior manufactured by Vital Scientific B. V. Netherlands. The procedure is according to the manufacturer's instruction. Urea and creatinine were analyzed using Randox test kits.

## 2.6 Liver Function Test

Serum biochemical indices routinely estimated for liver functions including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Bilirubin were determined using Randox diagnostic kits.

## 2.7 Alpha-amylase Activity

Alpha-amylase (Single Reagent) – GALG2-CNP Test Kit was used to assay for the inhibitory effect of the extract on the alpha-amylase enzyme. Alpha-amylase catalyzes the hydrolysis of 2-chloro-4-nitrophenyl-1-galactopyranosyl-maltoside (GALG2-CNP) to glucose polymers and p-nitrophenyl oligosaccharide at short chain producing 2-chloro-4-nitrophenol (CNP). The increased extinction was measured by spectrophotometry at 405nm. Alpha-amylase is calculated using the following formula:  $\Delta E = A_2 - A_1$ , Alpha amylase (U/L) =  $\Delta E \times 765$ .

## 2.8 Insulin Assay

Insulin (Enzyme Immunoassay Test Kit) was used to assay for the regeneration of the  $\beta$ -cells by the extract and production of insulin. Immunospec Insulin Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-insulin antibody for solid phase (microtiter wells) immobilization and another anti-Insulin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the insulin antibody coated microtiter wells. Then anti-Insulin antibody labeled with horseradish peroxidase (conjugate) is added. If Insulin is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the Insulin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 1 hour incubation at room temperature, the wells are

washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The colour development is stopped with the addition of stop solution. The colour changed to yellow and was measured spectrophotometrically at 450 nm. The concentration of Insulin is directly proportional to the colour intensity of the test sample.

The desired number of coated wells in the holder was secured. 50 $\mu$ l of Insulin standards, specimens and controls were dispensed into the appropriate wells. This was gently but thoroughly mixed for 10 seconds. 100 $\mu$ l of enzyme conjugate reagent was dispensed into each well. They were mixed gently for 30 seconds and then incubated at room temperature for 60 minutes. The incubation mixture was removed by emptying the plate content into a waste container. The microtiter plate was rinsed and emptied 5 times with 1 x washing buffer (300 $\mu$ l each well). The microtiter plate was stroke sharply onto absorbent paper to remove all residual water droplets. 100 $\mu$ l of TMB substrate reagent was dispensed into each well. It was gently mixed for 10 seconds and then incubated at room temperature in the dark for 20 minutes. The reaction was stopped by adding 100 $\mu$ l of Stop solution to each well. This was gently mixed for 10 seconds until the blue colour completely changed to yellow. The optical density was read at 450 nm with a microtiter plate reader within 15 minutes.

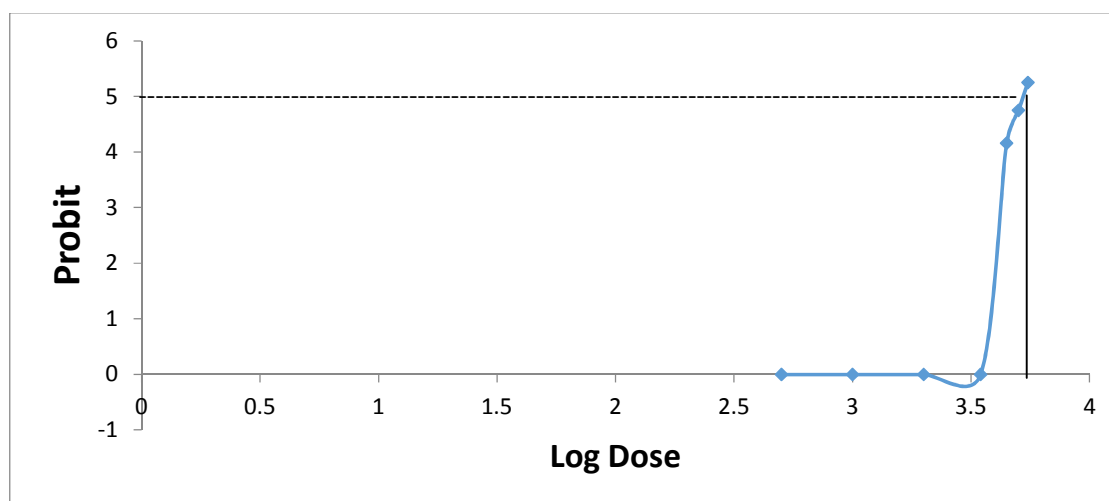
## 3. RESULTS

### 3.1 Acute Toxicity Study of the Ethanol Extract of Neem Leaf

The result of a 24-hour acute toxicity test of orally administered ethanol extracts of neem leaf in male albino rats is shown in Table 1. The groups that were administered with 0.5 g/kg, 1 g/kg and 2 g/kg body weight did not show any sign of toxicity. The group that was administered 3.5 g/kg body weight was slightly weak although no death was recorded. The group that received 4.5 g/kg body weight showed some signs of weakness with two deaths recorded. The groups that were administered 5 g/kg and 5.5 g/kg body weight were very weak with four and six deaths recorded respectively. The percentage mortalities were transformed to probit according to Finney's [20] method in order to extrapolate the  $\text{LD}_{50}$  from the graph of probit against log dose

**Table 1. Result of the acute toxicity study of the ethanol extract of neem**

(N=10) Groups	Dose (g/kg)	Log dose	No of death	% mortality	Probit	Remarks
A	0.5g	2.70	0	0	0	Normal
B	1g	3.00	0	0	0	Normal
C	2g	3.30	0	0	0	Normal
D	3.5g	3.54	0	0	0	Slightly weak
E	4.5g	3.65	2	20	4.16	Weak
F	5g	3.70	4	40	4.75	Very weak
G	5.5g	3.74	6	60	5.25	Very weak



**Fig. 1. LD<sub>50</sub> for orally administered ethanol extract of neem leaf**  
 Log dose at 50% = 3.70. LD<sub>50</sub> was derived by determining the antilog of log dose  
 Therefore  $\text{Log}^{-1} 3.7 = 5.0 \text{ g/kg}$ . LD<sub>50</sub> = 5.0 g/kg

(Fig. 1). The results obtained show that the LD<sub>50</sub> of the ethanol extract of neem leaf is 5 g/kg body weight.

### 3.2 Fasting Blood Glucose Profiles of Diabetic Rats Treated with Ethanol Neem Leaf Extracts

The result of twenty-eight (28) days treatment of diabetic rats with ethanol leaf extract of neem is shown in Table 2. Rats treated with ethanol extract of neem leaf showed a significant ( $p < 0.05$ ) reduction in blood glucose level from day 4. This reduction was consistent with continued administration and the effect appears to be dose-dependent (see Fig. 1). In all instances the effect achieved is significantly ( $p < 0.05$ ) better than that achieved with metformin; a standard drug used in the treatment of diabetes.

The reduction in the fasting blood glucose level of rats administered 400 mg/kg.b.w ethanol

extract of neem leaf were more pronounced compared with the groups administered 100 mg/kg b.w and 200 mg/kg b.w.

### 3.3 Modulation of Kidney Function in Diabetic Rats by Ethanol Neem Leaf Extracts

The result of the effect of treatment with ethanol leaf extract of neem on creatinine and urea is shown in Table 3. The disruptive effect of diabetes on the intracellular electrolytes, K<sup>+</sup> and Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> appear to be very minimal and is statistically insignificant. Administration of neem extracts appear to normalize this though the difference is not significant ( $p < 0.05$ ) compared with the diabetic untreated rats. The chloride ion concentration of the test groups was close to that of the normal non-diabetic rats. The result reveal that even the blood concentrations of Na<sup>+</sup>, urea and creatinine which were significantly ( $p < 0.05$ ) disrupted following the induction of diabetes were normalized after treating with neem leaf extract.

**Table 2. Fasting blood glucose levels of the rats used for antidiabetic studies measured at two days interval for a period of twenty-eight days of the study**

Time (Days)	Normal (Non-diabetic)	Untreated diabetic	100 mg/kg metformin	100 mg/kg ethanol extract	200 mg/kg ethanol extract	400 mg/kg ethanol extract
Initial b/f ind	90.40±10.36	98.40±22.58	89.80±9.311	76.60±11.01	91.80±11.14	79.20±5.020
Day 0	92.40±14.42	573.0±47.3b	533.4±110b	533.6±50.7b	555.4±88.9b	569.4±46.5b
Day 2	86.00±8.775	594.8±7.66b	554.8±67.8b	492.2±101bc	595.0±7.55b	535.0±94.4b
Day 4	76.20±7.050	600.0±0.00b	504.0±137b	436.0±91bc	465.4±129bc	343.4±86bc
Day 6	96.40±10.60	590.0±17.3b	491.4±94bc	345.0±77bc	246.8±122bc	182.2±94bc
Day 8	74.20±8.890	554.7±34.3b	375.6±158bc	350.8±104bc	293.8±197bc	192.2±87bc
Day 10	81.20±7.791	557.0±53.7b	308.8±134bc	190.5±137bc	368.6±200bc	286.8±89bc
Day 12	99.60±12.22	586.5±19.0b	339.6±165bc	157.8±110bc	157.8±128bc	223.8±86bc
Day 14	80.40±13.89	592.5±6.36b	335.2±163bc	217.8±109bc	179.8±67bc	211.2±68bc
Day 16	97.00±22.37	558.0±59.0b	339.4±58bc	183.3±126bc	156.5±71bc	99.00±39.7c
Day 18	91.00±11.77	534.5±51.6b	296.8±60bc	320.8±155bc	221.3±133bc	119.0±71.4c
Day 20	93.60±12.21	539.0±74.9b	287.8±143bc	286.5±75bc	255.0±96bc	197.8±112bc
Day 22	86.00±12.32	521.0±36.7b	307.4±176bc	258.0±211bc	211.8±138bc	166.2±112bc
Day 24	83.20±13.65	580.5±3.53b	321.2±159bc	282.4±198ac	198.8±87bc	181.0±109bc
Day 26	86.80±5.450	587.0±12.7b	262.6±255bc	207.8±145bc	184.0±99bc	145.8±97bc
Day 28	80.60±6.269	563.5±26.1b	253.8±119bc	206.3±169bc	177.0±156bc	143.8±79bc

<sup>a</sup>significant reduction with respect to normal control; <sup>b</sup>significant increase with respect to normal control; <sup>c</sup>significant reduction with respect to diabetic untreated control; <sup>d</sup>significant increase with respect to diabetic untreated control

The restorative effect of neem leaf extract in these instances are not dose-dependent and compared favourably with the effect of metformin.

### 3.4 Modulation of Liver Function in Diabetic Rats by Ethanol Extract of Neem Leaf

The result of the effect of treatment on the liver function indices (aspartate transaminase, alanine transaminase, alkaline phosphatase and bilirubin) is shown in Table 4. It is apparent from the result of untreated diabetic group that diabetes altered the liver function of rats significantly ( $p < 0.05$ ) leading to raised levels of bilirubin and liver function enzymes indicators. It is observed that neem leaf extract tried to reset these abnormalities in a dose-dependent manner with higher doses exerting better restorative effect. In terms of resetting liver function indices upset in diabetes, metformin appears to have done better than neem leaf extract.

### 3.5 Result of Alpha Amylase Assay

The result of the effect of treatment with the ethanol leaf extract of neem leaf on alpha amylase activity is shown in Table 5. The serum alpha-amylase activity of the extract treated

groups decreased significantly ( $p < 0.05$ ) compared with the diabetic untreated group and the non-diabetic group. The decrease in the serum alpha-amylase activity in the groups administered graded doses (100 mg/kgbw, 200 mg/kgbw and 400 mg/kgbw) of ethanol extract of neem leaf was dose-dependent. The group that was administered 100 mg/kgbw of metformin also showed a significant ( $p < 0.05$ ) reduction in the alpha amylase activity compared with the normal non-diabetic group.

### 3.6 Result of the Insulin Test

Result indicate that neem leaf extracts re-established the insulin levels in ever higher amounts than normal non-diabetic rats. This result suggests that the neem leaf extract has inducible effect on insulin production. The insulin level increased more in the group of rats treated with 200 mg/kg body weight ethanol extract compared with the rats treated with 100 and 400mg/kg body weight of the ethanol extract of neem leaf.

## 4. DISCUSSION

The 24-hour acute toxicity study of the orally administered ethanol extracts of neem leaf revealed that the median lethal dose ( $LD_{50}$ ) is

**Table 3. The effect of treatment with different doses of ethanol extract of neem leaf for a period of twenty-eight (28) days on the kidney function parameters**

Kidney Function Parameters	Normal (Non-diabetic)	Diabetic untreated rats	100mg/kg metformin	100 mg/kg ethanol extract	200 mg/kg ethanol extract	400 mg/kg ethanol extract
K <sup>+</sup> (mmol/L)	137.6±0.5477	133.5±2.121	140.8±1.924	138.8±0.9574	137.8±0.500	138.3±1.258
Cl <sup>-</sup> (mmol/L)	101.0±0.7071	99.50±0.703	101.0±0.701	100.8±0.5000	100.5±0.578	100.8±0.9574
Na <sup>+</sup> (mmol/L)	6.644±0.7014	8.055±0.219	6.840±0.316	6.473±0.2836	7.413±0.614	7.350±0.2317
HCO <sub>3</sub> <sup>-</sup> (mmol/L)	25.80±0.8367	27.50±0.707	26.20±0.837	26.25±0.9574	25.25±0.500	26.00±0.8165
Urea (mmol/dl)	26.60±0.8944	34.50±6.36b	26.20±1.30c	27.00±0.816c	25.50±1.29c	25.75±2.217c
Creatinine (mg/dl)	1.100±0.707	1.250±0.071	1.100±0.100	1.075±0.0957	1.100±0.082	1.125±0.0500

<sup>a</sup>significant reduction with respect to normal control; <sup>b</sup>significant increase with respect to normal control; <sup>c</sup>significant reduction with respect to diabetic untreated control; <sup>d</sup>significant increase with respect to diabetic untreated control

**Table 4. The effect of treatment with different doses of the ethanol extract of neem leaf respectively for a period of twenty-eight days on the liver function parameters**

Liver function parameters	Normal (Non-diabetic)	Diabetic untreated rats	100 mg/kg metformin	100 mg/kg ethanol extract	200 mg/kg ethanol extract	400 mg/kg ethanol extract
AST(U/L)	57.60±4.506	122.0±24.0b	56.00±11.4c	82.50±22.55	81.50±13.77	75.75±20.7c
ALT(U/L)	32.20±4.919	52.50±7.77b	34.00±8.06c	38.75±6.238	34.25±2.98c	30.25±2.63c
ALP(IU/L)	134.4±5.595	416.0±69.3b	137.0±5.91c	215.8±100c	173.0±69.07c	149.5±13.1c
Bilirubin (mg/dl)	0.630±0.078	1.8050±777b	0.942±0.15c	1.020±0.15c	1.270±0.282b	0.740±0.11c

<sup>a</sup>significant reduction with respect to normal control; <sup>b</sup>significant increase with respect to normal control; <sup>c</sup>significant reduction with respect to diabetic untreated control; <sup>d</sup>significant increase with respect to diabetic untreated control

**Table 5. The effect of treatment with different doses of ethanol extract of neem leaf respectively for a period of twenty-eight days on the alpha-amylase activity**

Control rats						
α- amylase activity(U/L)	Normal (Non-diabetic)	Diabetic untreated rats	100 mg/kg metformin	100 mg/kg ethanol extract	200 mg/kg ethanol extract	400 mg/kg ethanol extract
α-amylase	79.40±6.878	47.00±12.7a	33.60±9.0a	40.75±1.70a	38.45±17.2a	32.25±6.18a

<sup>a</sup>significant reduction with respect to normal control; <sup>b</sup>significant increase with respect to normal control; <sup>c</sup>significant reduction with respect to diabetic untreated control; <sup>d</sup>significant increase with respect to diabetic untreated control

**Table 6. The effect of treatment with different doses of ethanol extract of neem leaf for a period of twenty-eight days on the insulin level**

<b>Insulin level (ng/ml)</b>	<b>Normal (Non-diabetic)</b>	<b>Diabetic untreated rats</b>	<b>100 mg/kg metformin</b>	<b>100 mg/kg ethanol extract</b>	<b>200 mg/kg ethanol extract</b>	<b>400 mg/kg ethanol extract</b>
Insulin level	35.00±11.01	25.00±2.82 <sup>a</sup>	48.80±4.38 <sup>2d</sup>	40.50±11.82 <sup>d</sup>	45.25±15.20 <sup>d</sup>	41.50±8.58 <sup>2d</sup>

<sup>a</sup>significant reduction with respect to normal control; <sup>b</sup>significant increase with respect to normal control; <sup>c</sup>significant reduction with respect to diabetic untreated control; <sup>d</sup>significant increase with respect to diabetic untreated control



5.0 g/kgbw. A study based on rat model done by Raizada et al. [21] reported that neem leaf did not show any toxicity even at 5g/kgbw. According to Lorke [22], LD<sub>50</sub> values of orally administered extract observed to be 5 g and above simply show that the extract is not toxic.

The result of the antidiabetic study carried out for 28 days show that there was a significant ( $p < 0.05$ ) reduction in the fasting blood glucose level of the animals treated with the ethanol extract of neem leaf compared with the diabetic untreated group. The observed reduction faired even better compared with the animals administered 100 mg/kgbw metformin, a standard drug used in diabetes management. The reduction of blood glucose is dose-dependent becoming more prominent with increased doses of extract. This difference was more visible for the group of rats treated with 400 mg/kgbw of the ethanol extracts of neem leaf counting from the 6<sup>th</sup> day to the 28<sup>th</sup> day (Table 2). This observation agrees with the submission of Khosla et al. [17] that neem extract can control blood glucose.

Using routine diagnostic techniques it was observed that diabetes slightly altered electrolyte indicators of kidney function, K<sup>+</sup>, HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> albeit non-significantly but significantly increased blood Na<sup>+</sup>, urea and creatinine levels (Table 3). These results agree with the reports of Shravan et al. [23] who noted that diabetes disrupts serum urea and creatinine levels. This result can be attributed to the fact that diabetes causes the malfunction of the kidney thereby increasing the level of urea and creatinine in the blood. Administration of the ethanol extract of neem leaf at different doses re-established threshold values for K<sup>+</sup>, Cl<sup>-</sup>, Na<sup>+</sup>, HCO<sub>3</sub><sup>-</sup> and urea.

In like manner the major indicators for assessment of liver function, (AST, ALT, ALP and bilirubin) were all increased indicating serious perturbation of the liver (Table 4). The liver is highly susceptible to oxidative reactions as it is the main centre of detoxification of most of the substances in the body including xenobiotics. Induction of diabetes using STZ which caused a significant increase in serum markers of liver function (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total bilirubin) suggesting hepatic damage. Oral administration of ethanol extract of neem leaf at doses of 100 mg/kgbw, 200 mg/kgbw and 400 mg/kgbw

significantly ( $p < 0.05$ ) prevented increase in AST, ALT, ALP and TB levels of all the treated groups. These results are in line with the findings by Mohammed and Abubakar [24] who reported that the extracts of neem leaf have the potency of preventing liver damage in rats administered subcutaneously with 75 mg/kg CCl<sub>4</sub>. Comparing the level of these serum markers of liver function for the extract treated group with that of metformin, (a standard drug used for treatment of diabetes) did not show any significant difference. A close look at the indicators of these organ functions in diabetic rats treated with neem leaf extract suggests that the extract has an ameliorative effect that positively modifies the altered parameters towards normal values.

Plants contain different chemical constituents with potential for inhibition of  $\alpha$ -amylase and hence may be used as a therapy for diabetes mellitus [25]. The results obtained from the  $\alpha$ -amylase activity assay revealed a decrease in the  $\alpha$ -amylase activity of the diabetic rats treated with the ethanol extract of neem leaf (Table 5). This decrease in the activity of  $\alpha$ -amylase for the treated rats is significantly different ( $p < 0.05$ ) compared with that of the diabetic untreated rats. The presence of phytochemicals such as flavonoids, tannins and saponins which act as antioxidants in neem leaf extract may be responsible for its inhibitory activity on the  $\alpha$ -amylase enzyme. This is in line with the earlier report of Kazeem et al. [26] that neem leaf contains important phytochemicals which are responsible for its activity. The inhibition of carbohydrate hydrolyzing enzymes such as  $\alpha$ -amylase can be an important strategy in the management of type II diabetes.

The insulin level of the rats treated with different doses of the ethanol extracts increased ( $p < 0.05$ ) significantly compared with the diabetic untreated rats (Table 6). The diabetic untreated rats showed a serious decrease in their insulin level which expectedly is a result of the destruction of the  $\beta$ -cells of the pancreas by STZ. The increase in the insulin level of rats administered neem extract could be a result of regeneration from destruction of the  $\beta$ -cells of the pancreas. Most of the plant extracts seem to act directly on pancreas (pancreatic effect) and stimulate insulin level in blood [27]. Any substance which can directly act on the pancreas and stimulate insulin release will definitely have the capability of lowering the blood glucose level in the blood thereby ameliorating the effect of diabetes mellitus.

## 5. CONCLUSION

The ethanol extract of neem leaf can be effective in the management of diabetes and its complications as seen from the results of treatment for a period of twenty-eight days after the induction of diabetes. Ethanol extract of neem leaf is potent in lowering blood glucose level in STZ-induced diabetic rats by increasing the insulin level of the extract treated rats thereby regenerating the beta cells integrity. Its effect in lowering the fasting blood glucose level in STZ-induced diabetic rats compares favourably with metformin.

It modulates the essential biochemical parameters such as kidney functions, liver functions,  $\alpha$ -amylase and insulin level of diabetic rats favourably towards recovery and improved health. The observation made from this research reveals that the ethanol extract of neem leaf is effective in the treatment and management of diabetes mellitus.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" were followed. All experiments have been examined and approved by the ethics committee of Nnamdi Azikiwe University, Awka, Nigeria.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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