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The Modulatory effects of Methanolic Stem Bark Extract of *Acacia Nilotica* on Serum Level of CA 15-3, Liver and Kidney Biomarkers in Alloxan Induced Type-1 Diabetic Wistar Rats

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Abstract

The use of natural products as a means of treatment for many physiological threats such as diabetes is currently gaining momentum in Nigeria. Diabetes is a metabolic syndrome that afflicts an estimate of 433 million people and defects in liver function, kidney function and emergence of cancer are some of its complications. Hence this study was designed to evaluate the effect of *Acacia nilotica* on the Serum Level of CA 15-3**,** Liver and kidney function in Alloxan-induced type 1 diabetic Wistar Rats. A total of 28 rats were used in the study. Twenty-four (24) Rats of either gender was induced with 160mg/kg body weight of alloxan and left to develop diabetes for 72 hours after which there were divided into seven groups of four rats each: Group I: Normal Control, Group II: Diabetic Control, Group III, IV and V represent Diabetic rats treated with stem bark extract of *Acacia nilotica* (300mg, 600mg and 1200mg respectively). Group VI and VII represent Diabetic Rats treated with 250mg of metformin and 600mg of *Acacia nilotica* extract + 150mg of metformin respectively. The treatment was conducted for 14 days. At the end of the experiment, rats were fasted overnight and sacrificed. The blood samples were collected under chloroform anesthesia for the evaluation of liver and kidney biomarkers. The results indicated a statistically significant ($p<0.05$) increase in kidney and liver function parameters in diabetic rats when compared to normal rats except potassium, Total protein, albumin and direct bilirubin that were still normal. These findings indicate that *Acacia nolotica* possesses both renal and hepatoprotective effects on the kidney and liver. Further investigations should be conducted to elucidate the underlying mechanisms by which *Acacia nilotica* exerts these effects.

Keywords: Acacia Nilotica, $LD_{50,}$ Diabetes, Alloxan, Liver and Kidney function

Introduction

Diabetes mellitus is a metabolic disorder described by a persistent elevation in the blood glucose level. This persistent rise may either be triggered by the inability of β-cells of the islets found in the pancreas to secrete insulin or the failure of the liver/skeletal muscles cells to respond to insulin action (Ruud *et al.,* 2017). Body organ systems can be impacted by diabetes, which over time might result in catastrophic consequences. Diabetes complications can be microvascular or macrovascular in nature. Damage to the nerve system (neuropathy), the kidneys (nephropathy), and the eyes (retinopathy) are all examples of microvascular consequences. Cardiovascular disease, stroke, and peripheral vascular disease are examples of macrovascular consequences. Peripheral vascular disease can result in wounds that don't heal, gangrene, and ultimately an amputation for bruises or other injuries (American Diabetes Association, 2006). There are different types of diabetes, however, the two main types include: Type 1, is insulin-dependent or juvenile onset, its results from autoimmune beta-cell destruction in the pancreas and is characterized by a complete lack of insulin production. Type 2 is a non-insulin dependent or maturity onset, it develops when there is an abnormal increased resistance to the action of insulin and the body cannot produce enough insulin to overcome the resistance (ADA, 2006). It

is the most common form of diabetes accounting for about 90% of all cases (Hussein *et al*., 2016). Other specific but less common types include Gestational diabetes, which is a form of glucose intolerance that affects some women during pregnancy, a group of other types of diabetes caused by specific genetic defects of beta-cell function or insulin action, diseases of the pancreas, or drugs or chemicals (ADA, 2006).

Five – ten percent (5 to 10%) of all instances of diabetes are type 1 diabetes. Autoimmune, genetic, and environmental factors are among its risk factors. There are no known strategies to stop type 1 diabetes as of yet. Ninety (90) percent to 95% of all cases of diabetes that have been detected are type 2. This type of diabetes typically starts with insulin resistance, and because the body cannot make enough insulin to overcome the resistance, the pancreas may finally stop producing insulin altogether (IDF, 2017).

The classical symptoms of diabetes such as polyuria, polydypsia and polyphagia occur commonly in type 1 diabetes, which has a rapid development of severe hyperglycaemia and also in type 2 diabetes with very high levels of hyperglycaemia. Severe weight loss is common only in type 1 diabetes or if type 2 diabetes remains undetected for a long period. Unexplained weight loss, fatigue and restlessness and body pain are also common signs of undetected diabetes. Symptoms that are mild or have gradual development could also remain unnoticed (ADA, 2020).

Globally, there were approximately 8 million people with type 1 diabetes in 2021 (95% confidence interval: 8-1-8-8), of whom 1 million (18%) were under the age of 20, 5 million (64%) were between the ages of 20 and 59, and 1 million (19%) were 60 years or older. An estimated 35,000 undiagnosed people passed away within a year of the onset of symptoms in that year, out of a total of 0 to 5 million new cases that were diagnosed (median age of onset: 29 years) (Gabriel *et al*., 2021).

According to the International Diabetes Federation (IDF, 2021), over 25 000 children and adolescents in Africa under the age of 20 were predicted to have diabetes in 2019. Given the dearth of data from numerous nations and the high disease mortality, some argue that this figure understates the true burden of type 1 diabetes in sub-Saharan Africa (International Diabetes Federation, 2021).

The World Health Organization (2022) estimated the prevalence of Diabetes in Nigeria to be 4.3% and the prevalence is largely attributed to the lifestyle changes caused by urbanization and its results, industries producing unhealthy diets including sugar sweetened drinks, lack of exercise, tobacco use and harmful use of alcohol.

Diabetes can now be managed with a variety of therapies, including insulin therapy, medication, and nutrition therapy. Different types of glucose-lowering medications work through various processes to combat diabetes. Sulfonylurea and meglitinide drugs can stimulate insulin secretion, biguanides and thiazolidinediones can increase peripheral glucose absorption, alpha-glucosidase can delay the absorption of carbohydrates from the intestine, and biguanides can decrease hepatic gluconeogenesis (Bathaie *et al.,* 2012). Despite the considerable advancements in diabetes therapy over the past three decades, patient outcomes are still far from ideal. The treatments still have some drawbacks, such as toxicity, side effects, and medication resistance. For instance, 44% of patients have a loss of sulfonylurea efficacy after 6 years of therapy. Additionally, it is claimed that hyperlipidemia cannot be controlled by glucose-lowering medications. The adverse effects of medications and how they interact with one another in vitro must also be considered by medical personnel (IDF, 2017).

Today, a variety of treatments utilizing medicinal herbs are advised (Kooti *et al.,* 2015). According to Afrisham *et al.* (2015), the majority of plants have phytochemicals such as carotenoids, flavonoids, terpenoids, alkaloids, and glycosides, many of which have anti-diabetic properties. The anti-hyperglycemic effects that result from treatment with plants are often due to their ability to improve the performance of pancreatic tissue, which is done by increasing insulin secretions or reducing the intestinal absorption of glucose.

There is a long history of using plants to treat various human illnesses, including cancer, diabetes, malaria, hypertension, etc. Different plant parts, including leaf, stem, bark, root, and others, are utilized to prevent, alleviate symptoms, or restore anomalies to normal. Since the use of "herbal remedies" does not strictly follow the facts gathered via scientific methods, conventional medicine considers "herbal medicines" to be an alternate kind of treatment. Opium, aspirin, digitalis, and quinine are just a few examples of the pharmaceuticals that are routinely prescribed by doctors and have a long history of use as herbal treatments. Active substances derived from higher plants are used in modern medicine, and roughly 80% of these active components show a favorable association between their modern therapeutic usage and the traditional use (Sarkar *et al.,* 2015).

Acacia also called Babul is a medium-sized, thorny, virtually evergreen tree that can grow to a height of 20–25 m but may stay a shrub in unfavorable growing conditions (Vaarala, 2012).

Different antidiabetic drugs currently in use are associated with inadequate efficacy, expensive and have many side effects such as weight gain (Saidu, 2017).

Because of these and many others the attention of many patients has shifted to the use of alternative traditional remedies is increasing (Omagie and Agoreyo, 2014). Thus, this study is designed to properly investigate the safety of *Acacia nilotica* in the treatment and management of Diabetes. The aim of the study is to evaluate the effect of methanolic stem bark extract of *Acacia nilotica* on Serum Level of CA 15-3, kidney and liver biomarkers in alloxan induced diabetic rats.

Materials and Methods

Study Area

The study was conducted in the Department of Immunology, School of Medical Laboratory Science and Animal house of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto (UDUS).

Plant Collection and Identification

The fresh stem bark of *Acacia nilotica* was collected from the Garden of School of Medical Laboratory Science, UDUS. The Stem bark was identified and authenticated by Herbarium officer at Herbarium unit, Faculty of Pharmaceutical Sciences, UDUS. A voucher number of the plant specimen assigned was as follows: PCG/UDUS/FABA/0008.

Plant Preparation and Extraction

The stem bark of *Acacia nilotica* was allowed to shade dry. After drying, the stem bark was grinded into a coarse powder. The obtained powder was weighed using weighing balance and kept in an airtight container till further use. About 500g of the powder was soaked in 2 litres of methanol (2000mL). The mixture was vigorously shaken and allowed to stand for 48 hours at room temperature to ensure complete extraction. The liquid extract obtained was then filtered using Whatman No. 1 filter paper. The filtrate was placed in a hot air oven pre-set at a temperature of 50°C and a Semi-solid residue was obtained. The residue was then collected and weighed to be 105g. The residue was then transferred into a transparent wide mouthed screw-caped airtight universal sterile container labelled then stored in a Refrigerator at 4°C until required for use.

Ethical Approval

The study was approved by the Animal ethics committee of Usmanu Danfodiyo University Sokoto. The experiments were conducted in accordance with the institutional Animal Ethics Committee guidelines as well as internationally accepted practices for the use and care of laboratory animals.

Experimental Animals

A total of 45 Wistar albino rat of 8-12 weeks old, weighing between 90 -200 g of either gender (27 Females; 18 Males) were purchased from the Animal House of e Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto (UDUS). Animals were housed at Animal House of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto with alternating 12 hours of light and dark cycles at ambient temperature (25±5). Food and water were available to the Rats *ad libitum*. Animals were allowed for one week to acclimatize. The animals were maintained under

standard laboratory conditions and experimental protocol of the animal house was adapted.

Lethal Dose (Ld50) Determination

Lethal Dose (LD50) of the *Acacia nilotica* stem bark extract was carried out according to the methods of Lorke's (1983) in the animal house, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. Wistars rat of either gender was maintained under controlled conditions of temperature (20-25°C) and humidity (55%) were used for toxicity study. Lorke's method of LD50 consists of two phases.

The first Phase required nine animals. The nine animals were divided into three groups of three animals each. Each group of animals was administered different doses (10mg/kg bw, 100mg/kg bw and 1000mg/kg bw respectively) of *Acacia nilotica* stem bark extract. The animals were placed under observation for 24 hours to monitor their behavior (Alertness, feeding and weakness, etc. as well as mortality).

The second phase involved the use of three animals, which were distributed into three groups of one animal each. The animals were administered higher doses (1600mg/kg bw, 2900mg/kg bw and 5000mg/kg bw respectively) of *Acacia nilotica* stem bark extract and were observed for 24 hours for behavior as well as mortality (as shown in Appendix I).

LD50 was determined using Lorke's formula (Lorke, 1983) as follows:

$LD50 = \sqrt{a \times b}$

Where

a = Highest dosage at which death occurred in second phase,

b = The least dosage at which death occurred in the second phase

Experimental Design

After the acclimatization of the Animals, they were randomly divided into seven groups of four each. Group I (Normal control) received distilled water 10mL/kg b. w for 10 hours, orally. Group II (Positive control) received 160mg/kg b. w of alloxan intraperitoneally once followed by administration of distilled water orally for 14 days, Group III (Diabetic rats) received 160mg/kg b.w of alloxan intraperitoneally once followed by administration of 300mg/kg b. w of the extract for 14 days, orally, Group IV(Diabetic rats) received 160mg/kg b. w of alloxan intraperitoneally once, followed by administration of 600mg/kg b. w of the extract orally, for 14 days, Group V(Diabetic rats) received 160mg/kg b.w of alloxan intraperitoneally once, followed by administration of 1200mg/kg b.w of the extract orally, for 14 days, Group VI (Diabetic rats) received 160mg/kg b.w of alloxan intraperitoneally once, followed by administration of conventional drug (metformin) 250mg/kg b.w orally for 14 days and Group VII (Diabetic rats) received 160mg/kg b.w of alloxan intraperitoneally once, followed by administration of conventional drug (metformin) 150mg/kg b.w and 600mg/kg b.w of the extract orally for 14 days.

Table 1: Experimental animals grouping.

Groups N =4	**Treatment**
Group I (normal control)	Normal control rats received Distilled water orally
Group II (Positive control)	Diabetic control rats received distilled water orally only
Group III	Diabetic rats received 300mg/kg b.w of the extract
Group IV	Diabetic rats received 600mg/kg b.w of the extract
Group V	Diabetic rats received 1200mg/kg b.w of the extract
Group VI	Diabetic rats received conventional drug (Metformin) 250mg/kg b.w
Group VII	Diabetic rats received 600mg/kg b.w of of the extra ct + metformin 150mg/kg b.w

Induction of Type 1 Diabetes Mellitus

To induce experimental diabetes, Alloxan monohydrate was dissolved in saline solution (0.9% sodium chloride, pH 7) and was injected into rats as a single dose of 160 mg/kg intraperitoneally using diabetic syringe as recommended by Ajibola *et al.* (2014). The rats were placed on 10% glucose for next 24 hours to prevent hypoglycaemia (Misra and Aiman, 2012). After 72 hours, fasting blood glucose (FBG) was determined using On Call Plus one touch glucometer strips (Acon Laboratories) as described by Anees *et al.* (2007), those with glucose level >180 mg/dl were considered diabetic. The glucose level was assayed weekly to examine the effect of the extracts on the glucose level of the rats.

Blood Sample Collection and Processing

Twenty-four (24hrs) after the last treatment, the rats were fasted for 24 hrs before anesthetized in a glass jar containing cotton wool soaked with chloroform. About 5mL of blood were collected using a 5mL syringe. The blood samples were added into plain bottles for Biochemicals assay. The samples were centrifuged at 4000 revolution per minute (rpm) for 10 minutes, the serum obtained was transferred into cryovials using Pasteur pipette then labelled and stored in a deep freezer (at <-20 ^{0}C) until use.

Laboratory Analysis

The main Laboratory analysis that was conducted were:

Determination of Serum CA15-3

The serum concentration of CA15-3 was determined using quantitative sandwich ELISA techniques (Sunlong Biotech, China). The procedure was according to the manufacturer's instructions.

Liver Function Test

Some of markers of the liver functions that were evaluated includes: Alanine amino transferase (ALT), serum aspartate amino transaminase (AST), serum alkaline phosphatase (ALP),Total protein estimation, Albumin estimation, and Serum Total Bilirubin estimation, and Direct (conjugated Bilirubin) estimation.

Kidney Function Test

Some of markers of the kidney functions that were evaluated include: Serum Creatinine concentration, serum Urea concentration, and Electrolyte estimation (Sodium, Chloride and Potassium).

Data Analysis

The data obtained was analysed using statistical package for social science (SPSS) version 27 and the results were expressed as mean ± SEM. Group comparisons were made using one-way analysis of variance (ANOVA) and p-value of equal or less than 0.05 ($P \leq 0.05$) was considered significant. LSD test was used as post hoc test to compare some groups.

Results

Descriptive Analysis

In the study, 28 Wistar rats were used for determination of the effect of methanolic stem bark extract of *Acacia nilotica* on the expression of CA 15-3, liver and Kidney function parameters in alloxan induced diabetic rats. Animals aged 8-12 weeks old with weight between 120-200g were used. The study was conducted for 14 days. A one way between groups analysis of variance was conducted to explore the effect of methanolic stem bark extract of *A. nilotica on* liver and kidney functions in alloxan induced diabetic Wistar rats.

Presentation of Results

Phase one of Acute Oral Toxicity Studies

The result of phase 1 median lethal dose determination of methanolic stem bark extracts of Acacia nilotica after a single oral dose administration of 10mg/kg/bwt, 100mg/kg/bwt, 1000mg/kg/bwt for group I, group II and group III respectively. No death or any sign of toxicity were observed in all the three groups after 24 hours and up to end of the study (as shown in table 1)

Phase two of Acute Oral Toxicity study (Lethal Dose, LD_{50})

The results of phase II of acute oral toxicity study (median lethal dose determination) of the methanolic stem bark extracts of Acacia nilotica after a single oral dose administration of 1600mg/kg, 2900mg/kg, 5000mg/kg for group I, group II and group III respectively are depicted

in table 2. No mortality was documented in all groups after 24 hours.

Effect of methanolic stem bark extract of *Acacia nilotica* on serum expression of CA15-3 in alloxan induced diabetic rats.

Table 3 shows the serum expression of CA15-3 in Alloxan-induced diabetic Wistar rats treated with different doses of methanolic stem bark extract of *Acacia nilotica*, conventional drug and controls. After two weeks of treatment, there was statistically significant decrease in serum CA15-3 ($p<0.05$) expression in diabetic control, 300mg, 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+160mg of metformin (0.63±0.80, 1.01±0.30, 0.74±0.01, 0.77±0.08, 1.63±0.73 and 0.63±0.03 respectively) when compared to normal control (3.33±0.90). There was no significant ($p>0.05$) increase when diabetic control (0.63±0.80) was compared to 300mg, 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+160mg of metformin (1.01±0.30, 0.74±0.01, 0.77±0.08, 1.63±0.73 and 0.63±0.03 respectively). No significant ($p>0.05$) increase was observed when 300mg (1.01±0.30) was compared to 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+160mg of metformin (0.74±0.01, 0.77±0.08, 1.63±0.73 and 0.63±0.03 respectively), there was no significant difference when 600mg (0.74±0.01) was compared to 1200mg of the extract, 250mg of metformin and 600mg of extract+160mg of metformin (0.77±0.08, 1.63±0.73 and 0.63±0.03 respectively). There was no significant increase when 1200mg (0.77±0.08) was compared to 250mg of metformin and 600mg of extract+160mg of metformin (1.63±0.73 and 0.63±0.03 respectively). Between 250mg of metformin (1.63±0.73) and 600mg of extract+160mg of metformin (0.63±0.03), no statistically significant difference was observed.

Effect of methanolic stem bark extract of *Acacia nilotica* on liver function Biomarkers

Table 4 shows the results of the effect of intake of *Acacia nilotica* on liver function. There were statistically significant differences ($p<0.05$) on liver function parameters between normal control, positive control and groups treated with different doses of the extract and conventional drug(metformin) except for total protein, albumin and direct bilirubin that were non-significant ($p>0.05$) in all groups.

There was statistically significant increase ($p<0.05$) in AST values in Diabetic control, 250mg of metformin and 600mg of the extract+150mg of metformin (11.67±0.88, 10.33±0.88 and 11.33±0.88 respectively) whereas the AST values in 300mg, 600mg and 1200mg of the extract were not significantly ($p>0.05$) increased (7.00±1.15, 7.67±0.88 and 8.67±0.88) when compared to normal control (7.76±0.33).

In 300mg, 600mg and 1200mg methanolic stem bark extract of *A. nilotica*, there was significant decrease ($p<0.05$) in AST values (7.00±1.15, 7.67±0.88 and 8.67±0.88 respectively) when compared to diabetic control (11.67±0.88) and for 250mg of metformin and 600mg of the extract+150mg of metformin (10.33±0.88 and 11.33±0.88 respectively) there was no significant decrease ($p>0.05$). There was no statistically significant increase ($P>0.05$) between 300mg and 600mg and 1200mg (7.00±1.15, 7.67±0.88 and 8.67±0.88 respectively) however, there was statistically significant increase in 250mg metformin and 600mg of extract+150mg of metformin (10.33±0.88 and 11.33±0.88 respectively). In 1200mg, the was no statistically significant increase ($p>0.05$) in AST values (8.67±0.88), but there was statistically significant increase ($p<0.05$) in 250mg of metformin and 600mg of extract+150mg metformin (10.33±0.88 and 11.33±0.88 respectively) when compared to 600mg. Between 1200mg and 250mg of metformin, there was no statistically significant ($p>0.05$) increase in AST values (8.67±0.88 and 10.33±0.88 respectively) but there was significant increase ($p<0.05$) in 600mg of extract+150mg metformin (11.33±0.88). Likewise, there was no statistically significant decrease ($p>0.05$) in AST values between 250mg of metformin and 600mg of extract + 150mg of metformin (9.33±0.88 and 13.33±0.88 respectively).

For ALT, there was statistically significant increase ($p<0.05$) in ALT values of normal control (9.67±0.33) when compared to Diabetic control and 600mg of extract+150 mg of

metformin (13.67±1.20 and 13.33±0.88 respectively). However, there was no significant (p>0.05) increase in 300mg, 600mg, 1200mg of the extract and 250mg of metformin (9.67±0.88, 10.33±0.33, 9.00±1.15 and 9.33±0.88 respectively). In 300mg, 600mg, 1200mg and 250mg of metformin respectively (9.67±0.88, 10.33±0.33, 9.00±1.15 and 9.33±0.88 respectively), there was significant (p<0.05) decrease in ALT values except 600mg of extract+150mg of metformin (13.33±0.88) where there was no statistically significant(p>0.05) decrease in ALT values when compared to Diabetic control (13.67±1.20). Similarly, there was no statistically significant increase (P>0.05) between 300mg (9.67±0.88) and groups 600mg, 1200mg and 250mg of metformin (10.33±0.33, 9.00±1.15 and 9.33±0.88 respectively) however, there was statistically significant increase in 600mg of extract+150mg of metformin (13.33±0.88). Between 600mg (10.33±0.33) and 1200mg of extract and 250mg of metformin (9.00±1.15 and 9.33±0.88 respectively), there was no statistically significant increase (p>0.05) in ALT values but there was statistically significant increase (p<0.05) in ALT values between 600mg (10.33±0.33) and 600mg of extract+150mg metformin (13.33±0.88). Between 1200mg (9.00±1.15) and 250mg of metformin (9.33±0.88), there was no statistically significant (p<0.05) increase in ALT values but there was significant increase (p<0.05) in 600mg of extract+150mg of metformin (13.33±0.88). Likewise, there was statistically significant increase (p<0.05) in ALT values 250mg of metformin (9.33±0.88) and 600mg of extract + 150mg of metformin (13.33±0.88)

For ALP, there was statistically significant increase (p<0.05) in ALP in Diabetic control, 250mg of metformin and 600mg of extract+150mg of metformin (88.67±2.03, 86.67±2.96 and 91.67±1.33 respectively) when compared to normal control (78.33±2.19). However, there was no significant (p>0.05) increase in 300mg, 600mg, 1200mg of the extract and 250mg of metformin (84.00±5.13, 78.33±2.19 and 86.67±2.96 respectively) when compared to normal control (78.33±2.19). There was statistically significant decrease (p<0.05) in ALP values in Diabetic control (88.67±2.03) when compared to 600mg of extract (78.33±2.19) however, there was no significant decrease (p>0.05) in 300mg, 600mg, and 600mg of extract+150mg of metformin (84.00±5.13, 78.33±2.19 and 91.67±1.33 respectively), there was no significant (p>0.05) decrease in ALP values between 300mg of extract (84.00±5.13) and 600mg, 1200mg, 250mg of metformin and 600mg of extract +150mg of metformin (78.33±2.19, 86.33±1.45, 86.67±2.96 and 91.67±1.33 respectively). Between 600mg (78.33±2.19) and 250mg of metformin and 600mg of extract+150mg metformin (86.33±1.45 and 91.67±1.33 respectively), there was statistically significant increase (p<0.05) in ALP values but there was no statistically significant increase (p>0.05) in 1200mg (86.33±1.45). Between group 1200mg (86.33±1.45) and 250mg of metformin and 600mg of extract+150mg of metformin (86.67±2.96 and 91.67±1.33 respectively), there was no statistically significant (p>0.05) decrease in ALP values. When 250mg of metformin (86.67±2.96) was compared to 600mg of extract + 150mg of metformin (91.67±1.33), there was no statistically significant (p>0.05) decrease in ALP values.

For TB, there was statistically significant increase (p<0.05) between normal control (0.60±0.06) and all groups. There was statistically significant decrease (p<0.05) in TB values between Diabetic control (0.93±0.03) and all groups except group 300mg of extract (0.83±0.03) where there was no significant(p>0.05) decrease, there was no significant (p>0.05) decrease in TB values between 300mg of extract (0.83±0.03) and all groups. Between 600mg (0.77±0.03) and all groups, there was no statistically significant decrease (p>0.05) in TB values, there was statistically significant increase (p<0.05) in TB values between 1200mg of extract (0.73±0.03) and 250mg metformin (0.87±0.03) but there was no significant increase (p>0.05) in 600mg of extract + 150mg of metformin (0.79±0.06). There was no significant decrease (p>0.05) between 250mg metformin (0.87±0.03) and 600mg of extract + 150mg of metformin (0.79±0.06).

Effect of methanolic stem bark extract of *Acacia nilotica* on kidney function parameters

Table 5 shows the results of the effect of intake of *Acacia nilotica* on kidney function parameters. There were statistically significant differences

($p<0.05$) on liver function parameters between normal control, positive control and groups treated with different doses of the extract and conventional drug(metformin).

There was statistically significant ($p<0.05$) increase in urea level between normal control (4.63±0.23) and all groups. Urea level between diabetic control (13.30±0.83) and 300mg, 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+160mg of metformin (9.77±0.12, 8.53±0.15, 7.30±0.17, 6.13±0.24 and 6.00±0.76 respectively) was significantly reduced ($p<0.05$). Between 300mg (9.77±0.12) and 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+150mg of metformin (8.53±0.15, 7.30±0.17, 6.13±0.24 and 6.00±0.76 respectively), there was statistically significant decrease ($p<0.05$). in 600mg (8.53±0.15), there was statistically significant ($p<0.05$) decrease in urea level compared to 1200mg of extract, 250mg of metformin and 600mg of extract+160mg of metformin (7.30±0.17, 6.13±0.24 and 6.00±0.76 respectively). There was no statistically significant ($p>0.05$) decrease in urea level of 250mg of metformin (6.13±0.24) when compared to 1200mg of extract (7.30±0.17) but there was significant decrease when compared to 600mg of extract+150mg of metformin (6.00±0.76). Between 250mg of metformin (6.13±0.24) and 600mg of extract+150mg of metformin (6.00±0.76), there was statistically significant ($p<0.05$) decrease in urea level.

For serum creatinine concentration, there was statistically significant increase in diabetic control and 300mg of extract (0.93±0.03 and 0.93±0.03 respectively) when compared to normal control (0.73±0.03) but there was no significant increase ($p>0.05$) in 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+150mg of metformin (0.83±0.03, 0.83±0.03, 0.73±0.03 and 0.80±0.06 respectively). Between Diabetic control (0.93±0.03) and 300mg, 600mg and 1200mg of extract (0.93±0.03, 0.83±0.03 and 0.83±0.03 respectively), there was no statistically significant decrease ($p>0.05$) but there was significant ($p<0.05$) decrease in 250mg of metformin and 600mg of extract+150mg of metformin (0.73±0.03 and 0.80±0.06), when 300mg (0.93±0.03) was compared to 600mg and 1200mg of the extract (0.83±0.03 and 0.83±0.03 respectively), there was no statistically significant decrease ($p>0.05$) but there was significant ($p<0.05$) decrease in 250mg of metformin and 600mg of extract+150mg of metformin (0.73±0.03 and 0.80±0.06 respectively). There was no statistically significant ($p>0.05$) decrease in creatinine concentration between 600mg (0.83±0.03) and 1200mg, 250mg of metformin and 600mg of extract+150mg of metformin (0.83±0.03, 0.73±0.03 and 0.80±0.06 respectively). Similarly, there was no statistically significant decrease ($p>0.05$) in creatinine concentration between 1200mg (0.73±0.03) and 250mg of metformin and 600mg of extract+150mg of metformin (0.73±0.03 and 0.80±0.06 respectively). There was no statistically significant decrease ($p>0.05$) between 250mg of metformin (0.73±0.03) and 600mg of extract+150mg of metformin (0.80±0.06).

For serum sodium concentration, there was statistically significant ($p<0.05$) decrease in sodium level between normal control (145.33±0.88) and all groups. sodium level between diabetic control (126.67±0.88) and 300mg, 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+160mg of metformin (131.33±0.88, 135.33±0.88, 138.00±0.58, 141.33±0.88 and 141.67±0.88 respectively) was significantly ($p<0.05$) increased. Between 300mg (131.33±0.88) and 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+150mg of metformin (135.33±0.88, 138.00±0.58, 141.33±0.88 and 141.67±0.88 respectively), there was statistically significant increase ($p<0.05$) in sodium concentration. In 600mg (135.33±0.88), there was statistically significant ($p<0.05$) increase in serum level compared to 1200mg of extract, 250mg of metformin and 600mg of extract+160mg of metformin (138.00±0.58, 141.33±0.88 and 141.67±0.88 respectively). There was statistically significant ($p<0.05$) increase in sodium level of 250mg of metformin and 600mg of extract+150mg of metformin (141.33±0.88 and 141.67±0.88 respectively) when compared to 1200mg of

extract (138.00±0.58). Between 250mg of metformin (141.33±0.88) and 600mg of extract+150mg of metformin (141.67±0.88), there was no statistically significant ($p>0.05$) increase in sodium level.

For serum potassium concentration, there was no statistically significant ($p>0.05$) increase in diabetic control, 300g, 600g of the extract and 250mg of metformin (4.53±0.15, 4.43±0.17, 4.53±0.15 and 4.30±0.15 respectively) but there was significant ($p<0.05$) increase in 1200g and 600mg of extract+150mg of metformin (4.83±0.88 and 4.63±0.88 respectively) when compared to normal control (4.17±0.88). Between diabetic control (4.53±0.15) and other groups, there was no statistically significant increase ($p>0.05$). Between 300mg (4.43±0.17) and other groups, there was no significant ($p>0.05$) increase except in 1200mg (4.83±0.88) where there was significant ($p<0.05$) increase. There was no significant ($p>0.05$) decrease when 600mg (4.53±0.15) was compared to other groups. Between 1200mg (4.83±0.88) and 250mg of metformin (4.30±0.15), there was significant ($p<0.05$) decrease in potassium concentration (4.30±0.15) but there was no significant decrease ($p>0.05$) in 600mg of extract+150mg of metformin (4.63±0.88), there was no significant ($p>0.05$) decrease between 250mg of metformin (4.30±0.15) and 600mg of extract+150mg of metformin (4.63±0.88).

There was statistically significant ($p<0.05$) decrease in serum chloride concentration between normal control (103.0±1.15) and all groups except 600mg of extract+150mg of metformin where there was no significant ($p>0.05$) decrease (101.67±0.88), there was statistically significant ($p<0.05$) increase in chloride concentration in all groups compared to diabetic control (90.00±0.58). between 300mg (92.00±0.58) and other groups, there was significant ($p<0.05$) increase in chloride concentration. There was no significant ($p>0.05$) increase in chloride concentration when 600mg (95.67±1.20) was compared to 1200mg (95.67±1.20) but there was significant increase when compared to 250mg of metformin and 600mg of extract+150mg of metformin (99.00±0.58 and 101.67±0.88 respectively). Between 250mg of metformin and 600mg of extract+150mg of metformin (99.00±0.58 and 101.67±0.88), there was statistically significant ($p<0.05$) increase.

For serum bicarbonate concentration, there was statistically significant ($p<0.05$) decrease in bicarbonate level between normal control (26.67±0.33) and all groups. bicarbonate level between diabetic control (19.33±0.33) and 300mg, 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+160mg of metformin (20.67±0.33, 22.33±0.33, 24.00±0.00, 24.67±0.33 and 25.33±0.33 respectively) was significantly ($p<0.05$) increased. Between 300mg (20.67±0.33) and 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract+150mg of metformin (22.33±0.33, 24.00±0.00, 24.67±0.33 and 25.33±0.33 respectively), there was statistically significant increase ($p<0.05$) in bicarbonate concentration. In 600mg (22.33±0.33), there was statistically significant ($p<0.05$) increase when compared to 1200mg of extract, 250mg of metformin and 600mg of extract+160mg of metformin (24.00±0.00, 24.67±0.33 and 25.33±0.33 respectively). There was no significant ($p>0.05$) increase when 1200mg (24.00±0.00) was compared to 250mg of metformin (24.67±0.33) but there was significant increase when compared to 600mg of extract+160mg of metformin (25.33±0.33). there was no significant increase ($p>0.05$) in chloride concentration when 250mg of metformin (24.67±0.33) was compared to 600mg of extract+160mg of metformin (25.33±0.33)

Table 1: Phase I of Acute Oral Toxicity study (Lethal Dose, LD_{50}).

Grp	N	Dose	Mortality
Grp I	3	10mg/kg	**0**
Grp II	3	100mg/kg	**0**
Grp III	3	1000mg/kg	**0**

Key: **N** means numbers of rats

Table 2: Phase II Acute Oral Toxicity study (Lethal Dose, LD_{50})

Grps	N	Dose	Mortality
Grp I	1	1600mg/kg	**0**
Grp II	1	2900mg/kg	**0**
Grp III	1	5000mg/kg	**0**

Key: **N** means number of rats

Table 3: Effect of methanolic stem bark of Acacia nilotica on serum level of CA 15-3 expression

Groups	Number of Rats	CA15-3 serum level (pg/mL)
G1	4	3.33 ± 0.90^{a}
G2	4	0.63 ± 0.80^{b}
G3	4	1.01 ± 0.30^{b}
G4	4	0.74 ± 0.01^{b}
G5	4	0.77 ± 0.08^{b}
G6	4	1.63 ± 0.73^{b}
G7	4	0.63 ± 0.03^{b}
F-value		4.71
P-value		0.01

N=number of rats, G1=Negative Control, G2=Positive Control, G3=300mg/kg bw of extract, G4=600mg/kg bw of extract, G5=1200mg/kg bw of extract, G6=250mg/kg bw of metformin, G7= 600mg/kg bw of extract + 150mg/kg bw of extract). Values are expressed as mean ± SEM using Analysis of variance (ANOVA), mean values having different superscript in a column are significantly different CA15-3 = carbohydrate antigen 15-3, pg/mL= picograms per millitre.

Table 4: Effect of methanolic stem bark extract of *Acacia nilotica* on Liver Function

Groups N= 21	Number of Rats	TP (g/dL)	Alb (g/dL)	AST (u/l)	ALT (u/l)	ALP (u/l)	T.Bil (mg/dL)	D.Bil (mg/dL)
G1	4	68.67±0.33	38.33±0.33	7.76 ± 0.33^{a}	9.67 ± 0.33^{a}	78.33 ± 2.19^{a}	0.60 ± 0.06^{a}	0.23±0.03
G2	4	68.00±0.58	37.67±0.33	11.67 ± 0.88^{bc}	13.67 ± 1.20^{b}	88.67 ± 2.03^{b}	0.93 ± 0.03^{bd}	0.23±0.03
G3	4	67.33±0.88	38.00±0.58	7.00 ± 1.15^{a}	9.67 ± 0.88^{a}	84.00 ± 5.13^{ab}	0.83 ± 0.03^{bcd}	0.23±0.03
G4	4	67.33±1.20	37.33±0.33	7.67 ± 0.88^{a}	10.33 ± 0.33^{a}	78.33 ± 2.19^{a}	0.77 ± 0.03^{cd}	0.27±0.03
G5	4	68.00±0.58	38.00±0.00	8.67 ± 0.88^{ac}	9.00 ± 1.15^{a}	86.33 ± 1.45^{ab}	0.73 ± 0.03^{c}	0.23±0.03
G6	4	68.33±0.33	38.00±0.58	10.33 ± 0.88^{bc}	9.33 ± 0.88^{b}	86.67 ± 2.96^{ab}	0.87 ± 0.03^{bd}	0.27±0.03
G7	4	67.67±1.33	37.67±0.58	11.33 ± 0.88^{bc}	13.33 ± 0.88^{a}	91.67 ± 1.33^{b}	0.79 ± 0.06^{cd}	0.27±0.03
F-value		0.36	0.67	4.78	4.10	3.37	0.52	0.29
P-value		0.90	0.68	0.01	0.01	0.03	0.00	0.93

N=number of rats, G1=Negative Control, G2=Positive Control, G3=300mg/kg bw of extract, G4=600mg/kg bw of extract, G5=1200mg/kg bw of extract, G6=250mg/kg bw of metformin, G7= 600mg/kg bw of extract + 150mg/kg bw of extract. Values are expressed as mean ± SEM using Analysis of variance (ANOVA), mean values having different superscript in a column are significantly different, e3TP = Total Protein, Alb = Albumin, AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline Phosphatase, T.Bil = Total Bilirubin, D.Bil = Direct Bilirubin, u/l = International Unit/ Liter, mg/dL = Miligram per deciliter, g/dL = grams per decilite

Table 5: Effect of methanolic stem bark extract of *Acacia nilotica* on Kidney Function

Groups	No of Rats	Urea (mg/dL)	Creatinine(mg/dL)	Na^+ (mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)	$HCO3^-$ (mmol/L)
NC	4	4.63±0.23^a	0.73±0.03^a	145.33±0.88^a	4.17±0.88^a	103.0±1.15^a	26.67±0.33^a
PC	4	13.30±0.83^b	0.93±0.03^b	126.67±0.88^b	4.53±0.15^{abc}	90.00±0.58^b	19.33±0.33^b
G3	4	9.77±0.12^c	0.93±0.03^b	131.33±0.88^c	4.43±0.17^{ac}	92.00±0.58^b	20.67±0.33^c
G4	4	8.53±0.15^d	0.83±0.03^{ab}	135.33±0.88^d	4.53±0.15^{abc}	95.67±1.20^c	22.33±0.33^d
G5	4	7.30±0.17^e	0.83±0.03^{ab}	138.00±0.58^e	4.83±o.88^{bc}	95.67±1.20^c	24.00±0.00^e
G6	4	6.13±0.24^{ef}	0.73±0.03^a	141.33±0.88^f	4.30±0.15^{ac}	99.00±0.58^d	24.67±0.33^{ef}
G7	4	6.00±0.76^f	0.80±00.06^a	141.67±0.88^f	4.63±0.88^c	101.67±0.88^a	25.33±0.33^s
F-value		55.84	4.78	58.94	2.86	34.69	72.50
P-value		0.00	0.01	0.00	0.05	0.00	0.00

N=number of rats, G1=Negative Control, G2=Positive Control, G3=300mg/kg bw of extract, G4=600mg/kg bw of extract, G5=1200mg/kg bw of extract, G6=250mg/kg bw of metformin, G7= 600mg/kg bw of extract + 150mg/kg bw of extract. Values are expressed as mean ± SEM using Analysis of variance (ANOVA), mean values having different superscript in a column are significantly different, mg/dL = Miligram per deciliter, mmol/L =milli moles per liter, Na^+= Sodium, K^+= Potassium, Cl^- = chloride, $HCO3^-$= Bicarbonate.

Discussions

Diabetes mellitus is a metabolic disorder distinguished by a persistent elevation in the blood glucose level. This persistent rise may either be triggered by the inability of β-cells of the islets found in the pancreas to secrete insulin or the failure of the liver/skeletal muscles cells to respond to insulin action (Ruud *et al.,* 2017). Despite the considerable advancements in diabetes therapy over the past three decades, treatments still have some drawbacks, such as toxicity, side effects, and medication resistance. large number of plants have been recognized to be effective in the treatment of diabetes mellitus. The present study was conducted to assess the effects of methanolic stem bark extract of *A. nilotica* on serum expression of CA15-3, kidney and liver biomarker in alloxan induced diabetic rats.

The LD_{50} determination indicated that the administered doses of *A. nilotica* stem bark extract (300mg, 600mg and 1200mg) used in this study were not toxic to the experimental animals. This supports the safety profile of the extract and suggests its potential for further exploration as a therapeutic agent.

The current study indicated a significant decrease in serum level of CA15-3 expression in diabetic control and all test groups (300mg, 600mg, 1200mg of the extract, 250mg of metformin and 600mg of extract + 150mg of metformin) compared to normal control. After 14 days of treatment with methanolic stem bark of *A. nilotica*, no significant increase was observed when normal control (3.33±0.90) was compared to diabetic control (0.63±0.80) 300mg (1.01±0.30), 600mg (0.74±0.01), 1200mg of the extract (0.77±0.08), 250mg of metformin (1.63±0.73) and 600mg of the extract + 150mg of metformin (0.63±0.03). Although, diabetic rats treated with 250mg of metformin (1.63±0.73) and 300mg of the extract (1.01±0.30) showed slight increase in CA15-3 when compared to diabetic control (0.63±0.80). This finding disagrees with the findings of Xi-Yu (2022) who observed that cancer antigens such as CA15-3 returned to normal or within 2 weeks after good control of blood glucose. The decrease is thought to be due to destruction of pancreas which is among the organs responsible for production of different tumor markers (Xi-Yu, 2022).

The study indicated a significant increase in

AST, ALT, ALP and Total bilirubin in diabetic control rats when compared to normal control rats. However, following administration of *A. nilotica,* for 14 days, there was significant decrease in AST, ALT and ALP values in 300mg, 600mg and 1200mg of the extract when compared to diabetic control which is in accordance with the findings of Narayanan *et al*. (2013) who concluded that after administration of *A. nilotica* methanolic extract to the acetaminophen-treated animals, the levels of AST, ALT, and ALP were significantly ($P < 0.01$) reduced as compared to the acetaminophen alone treated animals (no treatment). The study is also in line with the finding of Manas *et al.* (2018) who observed that following treatment with *A. nilotica* leaves, overall diabetic complications were mitigated as reflected by lowered hepatic (ALT, AST). No significant difference was observed in total protein, albumin and direct bilirubin between treated groups and diabetic control when compared to normal control which is in disagreement with the findings of Narayanan *et al*., (2013) who discovered that Compared to the normal control, the total protein level was considerably ($P < 0.01$) lower in the acetaminophen-treated control group and administration of *A. nilotica* extract significantly increased the total protein level, whereas standard drugs silymarin and Liv52 also increased the level of total protein.

Administration of stem bark extract of *A. nilotica* for 14 days reduced total bilirubin in 600mg, 1200mg of extract, 250mg of metformin and 600mg of extract+150mg of metformin compared to diabetic control. This result is consistent with the finding of Narayanan *et al*. (2013) who observed that when *A. nilotica methanolic* extract was given to the animals receiving acetaminophen, the animals' levels of total bilirubin were considerably ($P < 0.01$) lower than those of the animals receiving acetaminophen alone (no therapy).

The leakage of large quantities of enzymes into the blood stream was associated with centrilobular necrosis of the liver. Similarly, in the study, increases in serum enzyme level of ALT, AST, and ALP after exposure to alloxan was observed and thereby confirms the hepatic structural damage. The levels of these enzyme levels have been restored up to normal range by *A. nilotica* treatment indicating its hepatoprotective action (Yadav *et al*., 2013).

The study indicated a significant increase in serum urea, creatinine and potassium levels in diabetic control rats when compared to normal control and significant decrease in sodium, chloride and bicarbonate levels when compared to normal control. Following treatment with methanolic stem bark extract of *A. nilotica* for 14 days, it was discovered that there was statistically significant decrease in serum levels of urea and creatinine in doses tested (300mg, 600mg and 1200mg) when compared to diabetic control which is in clash with the finding of Tanko *et al.* (2015) who observed that there was no statistically significant difference in serum levels of urea and creatinine in aqueous and crude fractions of *Acacia nilotica* plant on Wistar rats in the doses tested (500mg/kg body weight and 1000mg/kg body weight) between the control and test groups. The study is also in agreement with the finding of Manas *et al.*, (2018) who observed that following treatment with *A. nilotica* leaves, overall diabetic complications were mitigated as reflected by lowered Urea and creatinine levels. Based on the result obtained, it was observed that there was statistically significant increase in serum levels of sodium, chloride and bicarbonate ions in doses tested (300mg, 600mg and 1200mg) when compared to diabetic control which is in acceptance with the finding of Tanko *et al.,* (2015) in aqueous fraction of *A. nilotica.* Potassium level in tested doses (300mg, 600mg and 1200mg) was not significantly decreased compared to diabetic control which is in unison with the finding of Tanko *et al*., (2015) who concluded that there was no statistically significant difference in serum level of potassium ions in the doses tested (500mg/kg body weight and 1000mg/kg body weight) between the control and test groups. These significant differences occurred in a dose independent manner.

Conclusion

It is apparently clear from the results that, *Acacia nilotica* does not show any notable effects on the expression of serum CA 15-3, but it has a potent effects in the maintenance of serum level of

biochemical parameters, especially, there was a significant decrease in serum level of urea, and creatinine and a significant increase the level of sodium, chloride and bicarbonate ions. The level of all the liver enzymes were modulated and restored to normalcy. The extract has demonstrated reno and hepatoprotective effects on both the kidney and the liver.

Recommendations

Further comprehensive toxicological studies, including sub-chronic and chronic toxicity evaluations are essential to ensure safety of extract of Acacia nilotica before it can be recommended for use in Diabetic patients.

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