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RESEARCH ARTICLE

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# Effect of methanol crude leaves extract and aqueous fraction of *Acacia Nilotica* on lipid profile and liver enzymes on alloxan -induced diabetic wistar rats

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**ABSTRACT**

*The aim of this study is to determine the effect of methanol crude leaves extract and aqueous fraction of Acacia nilotica on liver enzymes and lipid profiles of alloxan induced diabetic Wistar rats. Diabetes was induced by a single intraperitoneal injection of alloxan dissolved in 0.9%v/v cold normal saline solution at a dose of 150 mg/kg body weight, after which the rats were randomly divided into six groups. Groups 1 served as control, Group 2, received insulin (6 i.u/kg), Group 3 received 500mg/kg of crude extract of Acacia nilotica. Group 4 received 1000mg/kg crude extract of Acacia nilotica, Group 5 received 500mg/kg aqueous fraction of Acacia nilotica and Group 6 received 1000mg/kg aqueous fraction of Acacia nilotica. The results obtained showed a statistically significant decrease ($p<0.05$) in levels of total cholesterol and triacylglycerol and an increase in the level of high density lipoprotein in groups the treated groups when compared with the untreated group group.. Also as regards to the liver enzymes there was a statistical significant decrease ($p<0.05$) in the levels of alanine aminotransferase alanine aspartate aminotransferase and Alanine phosphatase in groups treated groups when compared with the control untreated group. The phytochemical screening revealed the presence of tannins, saponins, flavonoids and steroids. The median lethal dose (LD50) in rats was calculated to be 2,154.1 mg/kg bodyweight for both the methanol crude leaves extract and aqueous fraction of Acacia nilotica.*

**Keywords** Diabetes Alloxan monohydrate, *Acacia nilotica* lipid profile, liver enzymes.

## INTRODUCTION

*Acacia nilotica* is a species of plant native to Africa, the Middle East and the Indian subcontinent and it is also currently an invasive species of significant concern in Australia. The plant tender young pods are eaten as vegetables, the roasted seeds serve as spice or are fermented to make alcoholic beverages and the boiled bark produced a coffee like beverage [1]. The seeds bark and leaves of the plant has a long history of medical uses that are still employed today. The bark of *Acacia nilotica* has been used for treating diarrhea, colds, hemorrhages, tuberculosis and leprosy in South Africa [2]. In Italian Africa, the wood is used to treat small pox. Egyptians, Nubians believe that diabetics may eat unlimited carbohydrates as long as they also consume powdered pods. Bruised leaves are also poultice into ulcers, the gum is used for cancers of ear, eye or testicles [3].

Lipid profile contains information about several different kinds of lipid that normally circulate in the blood. All of these lipid levels need to be evaluated in the context of other risk factors. If there are several other risk factors, a cholesterol level of 200 mg/dl might be considered a problem, while if there are no other risk factors, it might not be. Some of the other risk factors for cardiovascular disease are: smoking, high blood pressure, diabetes, age of over 45 years for males, age of over 55 years for females, and a family history of early heart disease. [4].

Liver function test are commonly used in clinical practice to screen for liver disease, monitor progression of known disease and the effect of potentiably hepatotoxic drugs. The most common liver function test include serum

aminotransferase, alkaline aminotransferase and bilirubin. Aminotransferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are sensitive indicators of liver cell injury and are helpful in recognizing hepatocellular diseases such as hepatitis. The elevated level of ALT is more specific indicator of liver injury[(5].

According to the lipid hypothesis, abnormally high cholesterol levels (hypercholesterolemia), that is higher concentration of LDL-C and lower concentration of functional HDL-C, are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease leads to myocardial infarction (heart attack), stroke and peripheral vascular disease, since higher blood LDL-C, especially higher LDL particles concentrations and smaller particle size, contribute to this process more than the cholesterol content of the LDL particles [6].

## MATERIALS AND METHODS

### Plant material

The leaves of *Acacia nilotica* was collected from Ahmadu Bello University, Zaria, Nigeria. The plant material was identified and authenticated by a taxonomist, in the herbarium section in the Department of Biological Science Ahmadu Bello University, Zaria, Nigeria, where a voucher specimen (No. 698) has been deposited for future reference.

### Extraction of Plant Material

The leaves extract of *Acacia nilotica* were air dried under the shade and grinded into free powder using morter and pestle. 200 grams of the powdered material was macerated in 100% methanol at room temperature for 24 hours. It was then filtered using a filter paper (Whatman size 1). The filtrate was then evaporated to dryness in an oven get the crude extract and kept in a sealed container at 4°C in a refrigerator until use.

Another 100 grams of the powdered material was macerated in 100% distilled water at room temperature for 24 hours. It was then filtered using filter paper (Whatman size 1). The filtrate was evaporated to dryness in an oven at 37°C. A brownish residue weighed 85grams was obtained and kept in a sealed container at 4°C in a refrigerator until use.

### Chemical used

Alloxan monohydrate was purchased from Sigma chemicals (St Louis U.S.A). The Biphasic Isophane Insulin AS Mixtard 30 HM Pen fill (Novo Nordisk AIS 2880 Bagsvaerd, Denmark. NAFDAC Reg no 04-1601). Accu-chek glucometer (Lifescan, Inc 2010 Milpitas, CA 95035, U.S.A) was use for the determination of blood glucose levels.

### Preliminary phytochemical screening

The fractions were subjected to preliminary phytochemical screening test for the presences of secondary metabolites according to the method described by [7]

### Acute toxicity studies ($LD_{50}$)

***Acute toxicity study:*** The lethal doses ($LD_{50}$) of the plant extract was determined by method [ 8] using 18 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the extract at doses of 10, 100 and 1000mg/kg body weight intraperitoneally. They were observed for 24 hours for signs of toxicity. In the second phase rats were divided into 4 groups of 3 rat each and were also treated with the crude methanol extract and the Aqueous fraction at doses of 1600, 2900 and 5000 mg/kg bodyweight (i.p).The median lethal dose ( $LD_{50}$ ) was calculated using the second phase.

### Experimental Animals

***Animals and Induction of Diabetes Mellitus:*** The animals were handled in accordance with international principles guiding the use and handling of experimental animals (United State National Institute for Health, 1985). Thirty Wistar rats of both sexes weighing 120-150 g were used for the study of the effects of *extract* and fraction on the blood glucose levels of the animals. They were kept in standard cages at $250^{C}$ and 12 h light/dark condition in the animal room of the Department of Human Physiology, ABU, Zaria, Nigeria. The animals were fed on commercial feeds and were given water *ad libitum* .The animals were fasted from feeds for 12 hours before the commencement of each experiment, but were allowed water *ad libitum.* The rats assigned to the diabetic groups were injected with alloxan monohydrate dissolved in sterile cold normal saline at a dose of 150 mg/kg body weight intraperitoneally as reported by earlier by [9]. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were treated with 20% glucose solution intraperitoneally after 6 hours [10].The were kept

for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycaemia. After a period of three days the rats with blood glucose levels greater than 200mg/dl were considered diabetic and were used for the study.

***Experimental Design:*** The alloxan -induced diabetic Wistar rats were randomly assigned into five groups (1-5) of five

Group 1: Normal control rats and received distilled water orally
Group 2: Diabetic rats were administered insulin (6 iu/kg)
Group 3: Diabetic rats were administered 500mg/kg of crude extract of *Acacia nilotica*
Group 4: Diabetic rats were administered 1000mg/kg crude extract of *Acacia nilotica*
Group 5: Diabetic rats were administered 500mg/kg aqueous fraction of *Acacia nilotica*
Group 6: Diabetic rats were administered 1000mg/kg aqueous fraction of *Acacia nilotica* .

***Determination of blood glucose levels:*** All blood samples were collected by cutting the tail-tip of the rats. Blood samples for blood glucose determination were collected from the tail at intervals of 1, 3, 5, 7, 9 and 12 days. Determination of the blood glucose level was done by the glucose-oxidase principle [11] using the ONE TOUCH Basic (Lifescan, Milpitas, CA) instrument and results were reported as mg/dl[12] .

**Collection and Preparation of Sera Samples**

Blood samples were drawn from the heart of animals via cardiac puncture after they have been fasted for 16-18 h. Blood samples were collected in plain tubes and were allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 3000 rpm for 10 min and the supernatant (serum) collected were then subjected to liver enzymes assay.

**Determination of Serum Total Cholesterol**

The serum level of total cholesterol (TC) was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of [13]. 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 $^0$C after mixing and the absorbance of the sample ($A_{sample}$)and standard ($A_{standard}$)was measured against the reagent blank within 30 minutes at 546 nm. The value of TC present in serum was expressed in the unit of mg/dl.

TC concentration = A *sample* /A *standard x* 196.86 mg/

**Determination of Serum Triacylglycerol**

The serum triacylglycerol level was determined after enzymatic hydrolysis of the sample with lipases as described by method of [14]. 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 $^0$C after mixing and the absorbance of the sample ($A_{sample}$ )and standard ( $A_{standard}$) was measured against the reagent blank within 30 minutes at 546 nm. The value of triglyacylglycerol present in the serum was expressed in the unit of mg/dl.

TGL concentration = A *sample*/A *standard x* 194.0 mg/dl

**Determination of Serum High-Density Lipoprotein Cholesterol**

The serum level of HDL-C was measured by the method of [15]. Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in the unit of mg/dl.

**Determination of Liver Enzyme Assay**

Blood sample were collected via cardiac puncture, which were centrifuge to get serum for liver enzymes assay. This include; Alkaline phosphatase, Alanine aminotranferase and Aspartate aminotranferase using [16].

***Statistical analysis:*** Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. Values of $p<0.05$ were considered as significant or less were taken as significant[17] .

## RESULTS

Preliminary phytochemical screening of the plant *Acacia nilotica revealed* the presence of flavonoids, tannins, saponins, cardiac glycosides, reducing sugars, glycosides, steroids and triterpenes.

***Acute toxicity study (LD50) :*** The sign of toxicity were first noticed after 12-18 hours of treatment. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake, and prostration after 12 hours of treatment. The median lethal dose ($LD_{50}$) in rats was calculated to be 2,154 mg/kg body weight for both crude and aqueous fraction of the *Acacia nilotica.*

**Table 1: Effects of crude extract and aqueous fraction of *Acacia nilotica* on serum lipid profile of alloxan induced diabetic Wistar rats**

| Treatment Given | Serum total cholesterol (mg/dl) | Serum triacylglycerol (mg/dl) | Serum high density lipoprotein (mg/dl) |
|---|---|---|---|
| Diabetic control | 84.17 ± 2.77 | 169.7 ± 8.54 | 62.9 ± 5.89 |
| Biphasic insulin (6.I.U/kg) | 58.1 ± 3.38[a] | 152.2 ± 26.6 | 86.5 ± 5.99 [a] |
| 500 mg/kg crude extract | 48.6 ± 3.99 [a] | 115.3 ± 7.99 [a] | 88.7 ± 1.07 [a] |
| 1000 mg/kg crude extract | 61.3 ± 4.69 [a] | 133.6 ± 8.21 [ns] | 91.7 ± 1.80 [a] |
| 500 mg/kg Aqueous fraction | 55.3 ± 2.54 [a] | 122.0 ± 1.73 [a] | 91.2 ± 2.49 [a] |
| 1000 mg/kg Aqueous fraction | 50.7 ± 1.55 [a] | 124.9 ± 2.54 [a] | 89.7± 3.14 [a] |

*P < 0.05 is statistically significant when compared to the control group while ns= significant.*

Table 1: shows the mean serum lipid profile values of control and experimental groups of the crude extract and the aqueous fraction Data generated revealed that the serum total cholesterol and serum triglyceride levels were significant decrease ($p<0.05$) in groups that received the crude extract and aqueous fraction when compared to untreated diabetic control group. However, the serum high density lipoprotein significant increase ($p<0.05$) in groups treated with crude and aqueous fraction when compared to untreated diabetic control group.

**Table 2: Effects of crude methanol extract and aqueous fraction of *Acacia nilotica* on serum liver enzymes of alloxan induced diabetic Wistar rats**

| Treatment Given | ALT (U/L) | AST (U/L) | ALP(U/L) |
|---|---|---|---|
| Diabetic control | 91.5± 2.17 | 201.3 ± 15.4 | 86.8 ± 2.56 |
| Biphasic insulin (6.I.U/kg) | 58.7 ± 5.80[a] | 135.5 ± 375 | 22.9 ± 1.18 [a] |
| 500 mg/kg crude extract | 50.3 ± 3.54 [a] | 118.2 ± 6.07 | 43.7 ± 3.64 [a] |
| 1000 mg/kg crude extract | 53.0 ± 2.27 [a] | 128.0 ± 4.54 | 50.3 ± 7.19 [a] |
| 500 mg/kg Aqueous fraction | 47.5 ± 5.69 [a] | 112.0 ± 7.35 [a] | 39.4± 2.02 [a] |
| 1000 mg/kg Aqueous fraction | 71.0 ± 8.91 [a] | 132.5 ± 13.3 [a] | 53.6± 4.42 [a] |

*P < 0.05 is statistically significant when compared to the control group while ns= significant.*

Table 2: Table 2: Effects of crude methanol extract and aqueous fraction of *Acacia nilotica* on serum liver enzymes of alloxan induced diabetic Wistar rats. Data generated revealed that were significant decrease ($p<0.05$) in the liver enzymes in groups that were treated with the crude extract and aqueous fraction when compared to untreated diabetic control group..

Diabetes mellitus is one of the major metabolic disorders and leads to abnormalities in lipids, carbohydrates and protein metabolism [18]. As regards to the crude and aqueous fraction, there was a significant increase ( $p<00.5$) in the triglyceride ,cholesterol and high density lipoprotein when compared with the control untreated group as showed in table 1.The present study indicated that *Acacia nilotica* crude methanol extract and aqueous fraction treatment increases the lipid profiles and liver enzymes of treated diabetic wistar rats. Tannins and polyphenols are reported to have antidiabetic effects [19]. And it is known that *Acacia nilotica* pods are rich in these substances [20; 21]. So, it can be concluded that *Acacia nilotica* methanol crude extract and fraction showed an antidiabetic effect because of the tannins and polyphenols therein. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in alloxan-induced diabetic rabbits [22]. High-density lipoprotein levels are decreased in type 2 diabetic patients and that ultimately leads to atheromatous disease [23]. From our results there was a decrease in the levels of cholesterol, triacylglycerol when compared with the control. While there was an increase in HDL levels when compared with the control untreated group.

Diabetes mellitus is the commonest cause of liver failure and hepatomegaly [24]. This study showed that there was a significant decrease ($p<0.05$) in the liver enzymes ( ALT,ALP and AST) levels in the groups treated with the methanol crude extract and aqueous fraction of *Acacia nilotica* when compared with the control untreated group. This may also decrease the risk of liver failure associated with Diabetes mellitus. The results are in agreement with the work of [22] who reported hypolipidemic effect of *Acacia nilotica* extract on diabetic induced animals.

## CONCLUSION

*Acacia nilotica* leaves methanol crude extract and aqueous fraction produces has hypolipidemic and hepato-protective effects on alloxan-induced diabetic rats.

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