

Effects of Ethyl acetate and n-Butanol Fractions of *Acacia nilotica* methanol Leaves Extract on Lipid profile and Liver Enzyme of Alloxan-Induced Diabetic Wistar rats

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ABSTRACT

This study aimed to evaluate the effects of ethyl acetate and n-butanol fractions of *Acacia nilotica* methanol leaves extract on lipid profile and liver enzyme on alloxan induced diabetic rats. 30 Wistar rats of both sexes were used for the study. The rats were divided into six groups with five rats in each group. The diabetic rats were treated with n- butanol and ethyl acetate for a period of 12 days. After which the animals were sacrificed and blood serum sample were taken from all the groups for the assessment of lipid profiles and liver enzymes. As regards to the lipid profile there was a significant decrease ($P<0.05$) in the triglyceride and cholesterol level in ethyl acetate treated group with 50 and 100 mg/kg , while, there was also a significant increase in the levels of high density lipoprotein when compared with the control untreated group. Also there was a significant decrease ($P<0.05$) in ALT, AST and ALP levels in ethyl acetate fraction treated group with 50 and 100 mg/kg when compared with the control untreated group. In relation to the n-butanol fraction at the two doses tested 100 and 200 mg/kg there was no significant change in the levels of triglyceride when compared with the control untreated. However there was decrease in the levels of cholesterol ($p<0.05$) and a significant increase in the levels of high density lipoprotein when compared with the control untreated. There was a significant decrease ($P<0.05$) in the levels of ALT, AST while there was no significant change in the level of ALP treated with the n-butanol fraction when compared with the control untreated group. The phytochemical screening revealed the presences of saponin, flavonoid, tannin and alkaloid. The median lethal dose (LD50) of the ethyl acetate in mice was calculated to be 471.2 mg/kg *b.w* and n-butanol is 774.5 mg/kg *b.w*. This results suggest that the Ethylacetate and n-butanol fractions of methanol leaves extract of *Acacia nilotica* has anti-hyperlipidemic and hepatoprotective effect on alloxan induced diabetic rats.

INTRODUCTION

Lipid profile assay forms one of the special investigations in clinical biochemistry laboratories worldwide. The assay is becoming of increased importance in many third world countries like ours because of increased cases of hypertension, diabetes mellitus, renal diseases, and other disease conditions. Lipid profile assay has found useful application in monitoring of patients with diabetes mellitus (Anaja *et al.*, 1995; Ferreira *et al.*, 1996, Poirier *et al.*, 1996) and in management of patient with coronary artery disease (Law *et al.*, 1994, Low *et al.*,

1996) as well as malnutrition (Ogunkeye and Ighogboja, 1992; Akuyam *et al.*, 2008). Abnormal lipid levels, especially elevated total cholesterol, low density lipoprotein and triglyceride and decreased high density lipoprotein cholesterol contribute additional risk to patients with diabetes mellitus. The occurrence of liver disease and raised liver enzymes indicate the severity of hepatic injury (Bidhan *et al.*, 2011). Disturbance of liver function in some patient with diabetes is well recognized. The liver plays a central and crucial role in the regulation of carbohydrate metabolism. Its normal functioning is essential for maintenance of blood glucose level and a continued supply to the organs that require a glucose energy source (West *et al.*, 2006). Liver function test are commonly used in clinical practice to screen for liver disease, monitor Progression of known disease and monitor the effect of potential

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hepatotoxic drugs. The most common liver function test include serum amino transferase, alkaline amino transferase, bilirubin. Amino transferase such as alanine amino transferase (ALT) and aspartate amino transferase (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 a more specific indicator of liver injury. Both enzymes are released into the blood in increasing amounts when the liver cell membrane is damaged (Pratt *et al.*, 1999).

Acacia nilotica is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India. It has been introduced in China, the Northern Territory and Queensland in Australia (where it is considered to be a pest plant of national importance), in the Caribbean, Indian Ocean islands, Mauritius, United States, Central America, South America and the Galápagos Islands). It has naturalized in several countries where it has been introduced as a medicinal, forage and fuel wood plant (Spicer *et al.*, 2007). The aim of this study is to determine the effect of Ethylacetate and n-butanol fractions of *Acacia nilotica* on lipid profile and liver enzyme on alloxan induced diabetic Wistar rats.

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 mortar and pestle. 200 grams of the powdered material was macerated in aqueous methanol (40 % distilled water and 60 % methanol) at room temperature for 24 hours. It was then filtered using a filter paper (whatman size 1). The filtrate was then partitioned with ethylacetate to get ethylacetate fraction which was evaporated to dryness in an oven at 37 °C. A greenish-brown residue weighing 8.5 grams (1.7% w/w) was obtained and kept in a sealed container at 4°C in a refrigerator until use. Another 200 grams of the powdered material was macerated in 40 % distilled water and 60 % methanol at room temperature for 24 hours. It was then filtered using filter paper (Whatman size 1). The filtrate was then partitioned with n-Butanol to get the n-Butanol fraction which was evaporated to dryness in an oven at 37°C. A brownish residue weighing 6.5 gram (1.3 % w/w) 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.

Phytochemical screening

The extract was subjected to preliminary phytochemical screening test for the presence of secondary metabolites according to the method described by Trease and Evan (1983).

Acute toxicity studies (LD₅₀)

The LD₅₀ determination for each of the fractions was conducted separately using modified method of Lorke (1983). For each of the fractions, the evaluation was done in two phases. In phase one, three groups of three rats each, were treated with 10, 100 and 1000 mg extract/kg body weight orally (ip) respectively. A fourth group served as control. The rats were observed for clinical signs and symptoms of toxicity within 24 hours.

Based on the results of phase one for the Ethylacetate fraction, fifteen fresh rats with three per group were each treated with 140, 225, 370 and 600 mg fraction/kg (ip) respectively. A fifth group served as control. Clinical signs and symptoms of toxic effects and mortality were then observed for seven days. Also based on the results of phase one for the n-Butanol extract, fifteen fresh rats with three per group were each treated with 200, 400, 600 and 800 mg fraction/kg (ip) respectively. A fifth group served as control. Clinical signs and symptoms of toxic effects and mortality were then observed for 72 hours

The LD₅₀ were then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose i.e. the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase.

Experimental Animals

A total of 30 Wistar rats of both sexes between the ages of 10 to 12 weeks old and weighed between 120-150grams were used for the study. The animals were housed in the Animal House, Department of Human Physiology, ABU, Zaria, Nigeria. The animals were randomized into experimental and control groups and were kept in polypropylene cages. The animals were fed on standard feeds (Vital feeds, Jos Nigeria) and allowed access to water *ad libitum*. The "Principle of laboratory animal care" (NIH publication No 85- 23)' guideline and procedures were followed in this study (NIH publication reserved 1985).

Induction of experimental diabetes mellitus

The animals were fasted for 16–18 hours with free access to water prior to the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of Alloxan monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9% ^{v/v} cold normal saline solution at a dose of 150 mg/kg body weight (Katsumat *et al.*, 1999). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution orally after 6h. The rats

were then kept for the next 24h on 5 % glucose solution bottles in their cages to prevent hypoglycemia (Dhandapani *et al.*, 2002). The diabetes was assessed in alloxan-induced rats by determining the blood glucose concentration 72 hours after injection of alloxan. The rats with blood glucose level above 200mg/dl were then selected for the study.

Experimental design

After the induction of diabetes, the alloxan induced diabetic wistar rats were randomly assigned into the following groupings;

Group 1 (n = 5) ----- Received normal saline i.p

Group 2 (n = 5) ----- Received insulin (6 i.u/kg body weight i.p)

Group 3 (n = 5) ----- were treated with 50mg/kg of ethyl acetate fraction of the extract of *Acacia nilotica* i.p

Group 4 (n = 5) ----- were treated with 100mg/kg of ethyl acetate fraction of the extract of *Acacia nilotica* i.p

Group 5 (n = 5) ----- were treated with 100mg/kg of n-butanol fraction of the extract of *Acacia nilotica* i.p

Group 6 (n = 5) ----- were treated with 200mg/kg of n-butanol fraction of the extract of *Acacia nilotica* i.p

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 Stein (1987). 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °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.

Determination of Serum Triglyceride

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of Tietz (1990). 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °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 triglyceride present in the serum was expressed in the unit of mg/dl.

Determination of serum high density lipoprotein- cholesterol

The serum level of HDL-C was measured by the method of Wacnic and Albers (1978). 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 serum liver enzymes

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 Reitman and Frankel method (1957).

Determination of serum aspartate aminotransferase (AST)

This was estimated by method as described by, Bergmeyer and Walefeld (1978). Briefly 100µL of the reagent was added to 100µL of the samples and then mixed and incubated at 37°C for 1min. the change in absorbance of the sample was measured per minute spectrophotometrically at the wavelength of 590nm as follows:

$$\text{AST activity (U/L)} = \Delta\text{AB}/\text{min} \times 1768$$

Determination of serum alanine aminotranferase (ALT)

This was estimated by method as described by, Bergmeyer and Walefeld (1978). Briefly 1000µL of the reagent was added to 100µL of the samples and then mixed and incubated at 37°C for 1min. the change in absorbance of the sample was measured per minute spectrophotometrically at the wavelength of 590nm as follows:

$$\text{AST activity (U/L)} = \Delta\text{AB}/\text{min} \times 1768$$

Determination of serum alkaline phosphatase (ALP)

This was estimated by method as described by, Bowers and Mc Comb (1966). Briefly 0.5mL of the reagent was added to 0.05mL (50µL) of the sample and then mixed and incubated at 37°C for 10min. the change in absorbance of the sample was measured per minute spectrophotometrically at the wavelength of 590nm as follows:

$$\text{Absorbance of sample} / \text{Absorbance standard} \times \text{Value of standard (U/L)}.$$

Statistical Analysis

Blood glucose levels were expressed in mg/dl as mean \pm SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. Values of $p < 0.05$ or less were taken as significant (Duncan *et al.*, 1977).

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the two fractions of *Acacia nilotica* extracts revealed the presence of saponin, flavonoid, tannin and alkaloid.

Acute Toxicity Studies

The signs of toxicity were first noticed after 4-5 hours of extracts administration. There were decreased locomotor activity and sensitivity to touch and pain. Also there was decreased feed intake, tachypnoea and prostration after 12-18 hours of fraction administration. Early deaths were recorded after 72 hours and late deaths 96 hours after fractions administration. The LD₅₀ were then

calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose i.e. the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase.

For the n-butanol fraction, there was 0% mortality at 1000mg/Kg and 33.3% mortality was the next highest lethal dose at 1600mg/Kg. The LD₅₀ of the n-butanol fraction was thus; $\sqrt{600 \times 800} = 774.5$ mg/Kg. For the Ethylacetate fraction, there was 0% mortality at 370mg/Kg and 33.3% mortality was the next highest lethal dose at 600mg/Kg. The LD₅₀ of the ethyl acetate fraction was thus; The LD₅₀ was thus; $\sqrt{370 \times 600} = 471.2$ mg/Kg.

Table 1: Effects of n-butanol and Ethylacetate fraction of *Acacia nilotica* extract on Serum lipid profile of alloxan induced diabetic Wistar rats.

Groups	Lipid Profile		
	Triglyceride (mg/dl)	Cholesterol (mg/dl)	High density lipoprotein (mg/dl)
Control	169.7 ± 8.54	84.17 ± 2.77	62.9 ± 5.89
Insulin (6 i.u/kg)	152.2 ± 26.6	58.1 ± 3.38 ^a	86.5 ± 5.99 ^a
n-butanol 100 mg/kg	127 ± 5.88 ^{ns}	60.28 ± 5.07 ^a	90.25 ± 2.65 ^a
n-butanol 200 mg/kg	132.5 ± 4.73 ^{ns}	52.75 ± 2.59 ^a	85.5 ± 5.17 ^a
Ethyl acetate 50mg/kg	124.5 ± 4.66 ^a	55.0 ± 3.58 ^a	94.1 ± 4.06 ^a
Ethylacetate 100mg/kg	110.0 ± 4.10 ^a	52.7 ± 1.79 ^a	89.0 ± 1.08 ^a

Values are mean ± SEM.

Values are statistically significant compared to control group at: ^aP<0.05 and ns= not significant.

Table 1 shows the effects of triglyceride (TRI) cholesterol (CHO) and high density lipoprotein of ethyl acetate and n-butanol fractions of *Acacia nilotica* methanol leaves extract. There was a significant decrease (P< 0.05) in the triglyceride and cholesterol levels when compared with control group in the two fractions tested. Whereas there was a significant increase in the HDL when compared with the control groups with the two fractions tested.

Table 2: Effects of n-butanol and Ethylacetate fraction of *Acacia nilotica* extract on Serum liver enzymes of alloxan induced diabetic Wistar rats.

Groups	Liver Enzymes		
	ALT(U/L)	AST(U/L)	ALP(U/L)
Control	91.5 ± 2.17	201.3 ± 15.4	86.8 ± 2.56
Insulin (6 i.u/kg)	58.7 ± 5.8 ^a	135.5 ± 3.75 ^a	22.9 ± 1.18 ^a
n-butanol 100 mg/kg	66.7 ± 6.36 ^a	129.2 ± 9.33 ^a	39.4 ± 7.27 ^a
n-butanol 200 mg/kg	54.5 ± 5.69 ^a	117.5 ± 4.57 ^a	59.2 ± 3.62 ^{ns}
Ethyl acetate 50mg/kg	56.0 ± 4.79 ^a	114.7 ± 3.54 ^a	52.7 ± 3.85 ^a
Ethylacetate 100mg/kg	47.5 ± 3.01 ^a	151.0 ± 2.48 ^a	49.7 ± 2.52 ^a

Values are mean ± SEM. Values are statistically significant compared to control group at: ^aP<0.05 and ns= not significant.

Table 2 shows the effects of alanine aminotranferase, aspartate aminotranferase and alkaline phosphatase of ethyl acetate and n-butanol fractions of *Acacia nilotica* methanol leaves extract. There was a statistically decrease (p<0.05) in ALT, AST and ALP when compared to the control.

DISCUSSION

Diabetes mellitus is one of the most common chronic diseases and is associated with hyperlipidemia and comorbidities such as obesity, hypertension. Hyperlipidemia is a metabolic

complication of both clinical and experimental diabetes (Saravanan and Pari, 2005). Alloxan, a beta cytotoxin, induces "chemical diabetes" (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues (Omamoto *et al.*, 1981). Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is potent inhibitor of lipolysis. Since it inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids (Loci *et al.*, 1994). During diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids in to the circulation (Agardh *et al.*, 1999). Increased fatty acids concentration also increases the β-oxidation of fatty acids, producing more acetyl CoA and cholesterol during diabetes. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats (Bopanna *et al.*, 1997). The increased concentration of free fatty acids in liver and kidney may be due to lipid breakdown and this may cause increased generation of NADPH, which results in the activation of NADPH dependent microsomal lipid peroxidation. A significant hypolipidemic effect of the effective drug is desirable in the management of diabetes and its complications (Luc and Fruchart, 1991).

Insulin treatment decreased the cholesterol, and triglyceride values significantly (P< 0.05) and increased HDL values significantly (P> 0.05), Insulin also produces a significant decrease in ALT, AST and ALP (P< 0.05) compared to the diabetic controlled rat.

Effects of *Acacia nilotica* fractions on serum triglyceride, cholesterol and HDL (mg/kg±SEM) levels in alloxan-induced diabetic rats after 21days of fraction are given in table 2. The serum levels of cholesterol (60.25±5.07 mg/kg and 52.75±2.59 at dose 100 and 200mg/kg respectively) of *Acacia nilotica* n-butanol fraction treatment were found to be significantly (P<0.05) decreased as compared to un treated alloxan-induced control). Whereas, the serum level of HDL (90.25±2.65mg/kg and 85.5±5.17 at dose 100 and 200mg/kg respectively) of fractions were found to be significantly (P<0.05) increased as compared to untreated alloxan-induced diabetic rats. The triglyceride (127.0 ± 5.88 mg/kg and 132.5±4.73 at dose 100 and 200mg/kg respectively) of acacia *nilotica* n-butanol fraction treatment were found to have no significance difference. The serum levels of triglyceride (124.5±4.66 mg/kg and 110.0±4.10 at dose 50 and 100mg/kg respectively) and cholesterol (55.00±3.58 mg/kg and 52.7±1.79 at dose 50 and 100mg/kg respectively) of *Acacia nilotica* ethylacetate fraction treatment were found to be significantly (P<0.05) decreased as compared to untreated alloxan-induced diabetic group (control). Whereas, the serum level of HDL (94.1±4.06 mg/kg and 89.0±1.08 at dose 50 and 100mg/kg respectively) of the fraction were found to be significantly (P>0.05) increased as compared to untreated alloxan-induced diabetic group (control). Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Claudia *et al.*, 2006). In

this study, administration of all doses of the ethyl acetate fractions of *Acacia nilotica* significantly reduced serum levels of cholesterol, triglyceride, and increased serum levels of high-density lipoprotein in alloxan induced diabetic Wistar rats while all doses of n-butanol showed no significance in triglyceride level but all doses of n-butanol showed a significant decrease in cholesterol level and significant increase in HDL. Many nutritional factors such as saponins and tannins have been reported to contribute to the ability of herbs to improve dyslipidemia (Nimenibouadia, 2003; Rotimi *et al.*, 2011). The significantly lowered cholesterol level may have contributed to the observed significant high serum high-density lipoprotein cholesterol in the animals.

The ethyl acetate fraction of *Acacia nilotica* significantly ($P<0.05$) decreased the raised parameters (triglyceride and cholesterol) to normal values. Also there was a significant ($P<0.05$) increased High density lipoprotein (HDL) levels when compared to control.

However, there was an increase in HDL level, which is a positive sign as HDL cholesterol known as good cholesterol which has relation to diminish the cardiac problems. Also n-butanol fraction of *Acacia nilotica* significantly ($P<0.05$) decreased the level of cholesterol when compared to control untreated. However there was no significant change in the levels of triglyceride (TRI) when compared to untreated control. Also the n-butanol fraction of *Acacia nilotica* has significantly ($P<0.05$) increased HDL cholesterol levels. The observed antihyperlipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis exerted by the fractions to the diabetic rats (Bopanna *et al.*, 1997). About 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol antheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease (Kwiterovich, 2000; James *et al.*, 2010). Therefore, the observed increase in the serum HDL-C level on administration of various doses of the fractions indicates that the fractions have HDL-C boosting effect.

The hepatoprotective effects of *Acacia nilotica* extract on serum ALT, AST and ALP levels in alloxan induced diabetic rats after 12 days of fractions administration. The serum levels of ALT (66.7 ± 6.36 and 54.5 ± 5.69 at dose 100mg/kg and 200mg/kg respectively), AST (129.2 ± 9.33 mg/kg and 117.5 ± 4.57 at dose 100mg/kg and 200mg/kg respectively) and ALP (39.4 ± 7.27 mg/kg at dose 100mg/kg) of *Acacia nilotica* n-butanol extract treatment were found to be significantly ($P<0.05$) decreased as compared to untreated alloxan-induced diabetic (control). but ALP (59.2 ± 3.62 at dose 200mg/kg) was found to be insignificant. The serum levels of ALT (56.0 ± 4.79 and 47.5 ± 3.01 at dose 50 and 100mg/kg respectively) AST (114.7 ± 3.54 and 151.0 ± 2.48 at dose 50 and 100mg/kg respectively) and (52.7 ± 3.85 and 49.7 ± 2.52 at dose 50 and 100mg/kg respectively) of *Acacia nilotica* ethylacetate fraction treatment were found to be significantly ($P<0.05$) decreased as compared to untreated alloxan-induced diabetic group (control) as shown in table 2.

Diabetes is the commonest cause of liver failure and hepatomegaly (Chatila *et al.*, 1996), which itself represents a huge and rapidly increasing problem. This study showed that the n-butanol and ethylacetate fractions of *Acacia nilotica* treatment decreased the elevated levels of ALT, AST and ALP which shows that *Acacia nilotica* may also decrease the risk of liver failure associated with Diabetes mellitus. In conclusion the results of the research clearly indicates the hypolipidemic and hepatoprotective effect of the ethylacetate and n-butanol of *Acacia nilotica* pod extract when administered to alloxan induced diabetic wistar rats.

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