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Evaluation of Root Extract of *Acacia nilotica* on Haematological and Lipid Profile in Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

Aim: *Acacia nilotica* root has been used for different medicinal purposes wherever the plant is found in Nigeria. This study was designed to evaluate the effects of *Acacia nilotica* aqueous root extract on hematological parameters and lipid profile in rats.
Place and Duration of Study: Research work carried out by the author in the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja and department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Abuja between June and December 2015.
Methods: Forty eight Wistar rats (180.00 ± 20 g) separated into 24 females and 24 males, were randomly divided into four groups of six rats per group. Group 1, served as the control and received 10 ml/kg body weight of distilled water, while rats in groups 2, 3 and 4 received extract at 125, 250 and 500 mg/kg body weight orally, respectively for 28 days. Blood was collected via cardiac puncture on day 29 (after the rats were sacrificed) into EDTA bottles (haematology profile) and plain bottles (lipid profile). Phytochemical screening and acute toxicity were also carried out.
Results: The extract at 500 mg/kg body weight, produced significant increase in red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb) and packed cell volume (PCV) compared to the control in male rats. In female rats, the dose of 250 mg/kg b.wt of the extract produced significant

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increase in Hb and PCV. There was significant decrease in total cholesterol and triglycerides at 500 mg/kg b.wt in both male and female rats.
Conclusion: The results of this study showed that aqueous root extract of *Acacia nilotica* can be used to correct anaemia by increasing PCV and also prevent hypercholesterolemia by reducing serum cholesterol.

Keywords: Acacia nilotica; anaemia; hypercholesterolemia; anti-lipidemia; phytomedicine.

1. INTRODUCTION

Acacia nilotica (Linn.) Willd. Ex Del. is a nitrogen-fixing plant that belongs to the family *Fabaceae*. It is a commonly used medicinal plant, found in Northern parts of Nigeria and some other African Countries; where it grows to 14-17 m in height and 2-3 m in diameter [1]. Phytochemicals with proven medicinal properties reported from different parts of the plant include, alkaloids, terpenes, tannins, saponins and phenolics [2,3]. The plant is a rich source of minerals such as potassium, iron, manganese and magnesium. It also contains some amino acids like cysteine, methionine, lysine, threonine and tryptophan [2,4].

The root is used as an aphrodisiac and antimalarial, while the gum obtained from the stem is used as an emulsifying agent and in preparation of drug formulations by pharmaceutical companies [5]. Studies on the ethylacetate extract of *A. nilotica* root [6] demonstrated antiplasmodial activity against chloroquine sensitive *Plasmodium berghei*. Also, previous study by Alli et al. [7] found aqueous root extract of *A*. *nilotica* to possess anti-malarial activity and this could account for its wide use by rural communities in Northern Nigeria. The plant is known to have strong antioxidant property, which could help to prevent and treat oxidative stress related diseases [2].

Hypercholesterolemia has been implicated as a strong risk factor for cardiovascular diseases. Persistent hypercholesterolemia could result in atherosclerosis, coronary artery diseases (CAD) and other oxidative stress related diseases [8].

The most common use of *Acacia nilotica* root in Northern Nigeria is for the treatment of malaria and based on the fact that most individuals suffering from malaria usually present with anaemia (manifesting as low packed cell volume), reduction in some haematological parameters and abnormal lipid profile, this study is aimed at determining the effects of repeated-dose administration of aqueous root extract of *A. nilotica* on haematological parameters and lipid profile in rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Root samples of *Acacia nilotica* were collected between 8 – 9 am in July, 2015 from Bamburu-Chaza village, Suleja, Niger State, Nigeria. The samples were identified and authenticated at the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, by a taxonomist (Mrs Grace Ugbabe). Reference specimen with voucher number NIPRD/H/6401 was prepared and deposited at the NIPRD herbarium.

2.2 Experimental Animals

Adult Wistar rats *(Rattus norvegicus,* 180.00 ± 20 g) of both sexes obtained from Animal Facility Center, NIPRD, Abuja, were used for this study. They were housed in well ventilated cages, fed with rat pellets and water *ad libitum*; and maintained under standard laboratory conditions (temperature 26 ± 2℃, with a 12–12 h light-dark cycle).

2.3 Reagents and Chemicals

Diagnostic assay kits for lipid profiles were obtained from Randox Laboratories Ltd., Antrim, United Kingdom. All other chemicals and reagents used were of analytical grade and prepared using distilled water.

2.4 Preparation of Aqueous Extract of *Acacia nilotica* Root

The root sample of *A. nilotica* was air dried to constant weight and pulverized using grinding machine. The powder was stored in an airtight container and kept in a cool dry place. Aqueous extraction was carried out following the cold maceration method described by Adzu and Salawu [9]. Four hundred grams of the powdered root of *A. nilotica* was soaked in 1 L of distilled water and kept on shelve for 72 hr with

intermittent shaking. The suspension was filtered after 72 hrs, with muslin cloth followed by filtration with Whatman filter paper (No.1). The filterate was freeze-dried using AMSCO/FINN-AQUA GT2 Freeze dryer (Germany) to obtain a chocolate colour residue weighing 58.9 g (14.7% yield).

2.5 Phytochemical Screening

The qualitative and quantitative screening of the extract for phytochemical constituents, were carried out using standard procedures [10].

2.6 Acute Toxicity Study

The acute toxicity of the aqueous extract of *A. nilotica* was evaluated in mice following the Organization for Economic Cooperation and Development Guidelines 423 [11]. Four groups, with three female mice in a group, received the aqueous extract orally, at doses of 50, 300, 2000 and 5000 mg extract/kg body weight, respectively, while the control group received 10 ml/kg b.w of distilled water. The animals were observed individually after dosing for signs of toxicity (changes in skin, fur, respiration, motor activity) once during the first 30 min, periodically during the first 24 h and thereafter daily for 14 days. The LD_{50} value obtained from this study was used in estimating the various graded doses used in the haematological and lipid profile study.

2.7 Grouping and Dosing of Animals for Haematology and Lipid Profile

Forty eight Wistar rats (180.00 ± 20 g) separated into 24 females and 24 males were randomly divided into four groups of six rats per group. Group 1, served as the control and received 10 ml/kg body weight of distilled water, while rats in groups 2, 3 and 4 received extract at 125, 250 and 500 mg/kg body weight orally, respectively for 28 days.

2.8 Haematological Profile Analysis

On day 29, rats from each group were anaesthetized with diethyl ether and blood samples (4 – 5 ml) were collected by cardiac puncture after the rats were aseptically cut opened. One portion of blood was collected into K^+-EDTA bottles for estimation of haematologic parameters using an automated haematology machine (Cell-Dyn™ Abbot, US), while the remaining portion was collected into plain bottles for determination of lipid profile.

The haematology autoanalyzer counts the red cells, lysed them to release the haemoglobin (Hb) and estimates Hb concentration using photometric analysis. The machine assumes that all nucleated cells are white blood cells and therefore counts white cells into lymphocytes and neutrophils, but eosinophils, monocytes and basophils are recorded as mixed cells [12].

2.9 Lipid Profile Assay

Total cholesterol determination in serum was done following the method described by Friedrickson et al. [13]. The determination of triacylglycerol concentration in serum was carried out following the method described by Fossati and Prencipe [14]. The determination of high density lipoprotein concentration was carried out following the method described by Albers et al. [15]. The method described by Demacker et al. [16] was used in the determination of low density lipoprotein concentration in the serum.

2.10 Statistical Analysis

Data obtained were analyzed using Graph pad prism version 5 and these were expressed as mean ± standard error of mean (SEM). The differences between means were compared using Analysis of Variance (ANOVA) followed by Dunnet's post hoc test. $P = .05$ was considered significant.

3. RESULTS

3.1 Phytochemical Constituents of Aqueous Extract of *A. nilotica* Root

The summary of the phytochemical constituents of aqueous extract of *Acacia nilotica* root, shown in Table 1, revealed that the extract gave positive reactions to Phenolics, tannins, alkaloids, anthraquinones, flavonoids, terpenes and sterols. The phytochemicals are listed in the order of decreasing concentration.

3.2 Acute Toxicity Study

The extract did not cause death or change in physical appearance and morphological characteristics in the treated animals throughout the 14-day observation period after single oral administration of 50, 300, 2000 and 5000 mg/kg doses of aqueous extract of *A. nilotica* in the acute toxicity study. The estimated oral median lethal dose (LD50) in mice was 5000 mg/kg body weight.

Table 1. Phytochemical constituents of aqueous extract of *A. nilotica* root

Phytochemicals	Quantity (mg/kg)
Phenolics	34.50 ± 1.55
Tannins	27.00 ± 1.75
Alkaloids	23.30 ± 1.86
Saponins	9.80 ± 0.89
Anthraquinones	4.70 ± 0.97
Flavonoids	0.50 ± 0.06
Terpenes	0.10 ± 0.05
Sterols	0.10 ± 0.05

n = 3 ± SEM

3.3 Effect of *A. nilotica* on Haematological Parameters

In male rats, the extract at 500 mg/kg body weight dose, produced significant increase ($p < 0.05$) in the levels of RBC, WBC, haemoglobin and PCV compared to the control. Similarly, all the three doses of the extract administered resulted in significant increase in platelets count when compared with the control. In female rats, the dose of 250 mg/kg b.wt of the extract produced significant increase in Hb and PCV, while WBC was significantly elevated at the dose of 500 mg/kg b.wt. Platelet count was significantly elevated at 125 and 250 mg/kg b.wt of the extract when compared with the control. Other haematological parameters were not significantly altered by different doses of the extract (Table 2).

3.4 Effect of *A. nilotica* on Total Cholesterol and Triacylglycerol

The extract at the dose of 500 mg/kg body weight produced significant decrease ($p < 0.05$) in serum total cholesterol and triacylglycerol in both male and female treated rats (Tables 3 and 4).

Table 2. Effect of aqueous extract of *A. nilotica* root on haematological parameters of rats

Parameters	Control (10 ml/kg b.wt dist. water)	125 mg/kg b. wt extract	250 mg/kg b.wt extract	500 mg/kg b.wt extract
Male				
RBC (× 10^{12}/L)	6.90 ± 0.16	6.70 ± 0.38	7.30 ± 0.57	8.40 ± 0.19 *
Hb (g/dL)	12.80 ± 0.25	12.40 ± 0.89	13.30 ± 0.93	14.50 ± 0.35*
PCV (%)	39.20 ± 0.64	37.80 ± 1.09	40.70 ± 1.23	41.30 ± 0.73*
MCV (fL)	62.00 ± 0.69	60.30 ± 1.18	61.00 ± 0.95	61.00 ± 0.82
MCH (pg)	18.50 ± 0.30	18.50 ± 0.23	18.20 ± 0.48	18.20 ± 0.31
MCHC (g/dL)	32.60 ± 0.55	32.80 ± 0.46	32.60 ± 0.72	32.70 ± 0.35
WBC (×10^9/L)	10.80 ± 2.16	10.90 ± 1.83	10.10 ± 1.20	14.80 ± 2.57*
PLT (×10^9/L)	625.00 ± 58.0	732.00 ± 39.5*	714.00 ± 49.7*	688.00 ± 42.9*
LYMPH (%)	84.50 ± 0.10	83.80 ± 2.34	84.80 ± 1.65	84.00 ± 2.67
NEUT (%)	12.70 ± 0.98	13.70 ± 0.83	14.20 ± 1.48	13.70 ± 1.56
MXD (%)	2.80 ± 0.41	3.00 ± 0.84	2.40 ± 0.50	2.60 ± 0.48
Female				
RBC (× 10^{12}/L)	6.00 ± 0.28	6.60 ± 0.40	6.90 ± 0.48	6.30 ± 0.22
Hb (g/dL)	11.60 ± 0.36	12.30 ± 0.75	12.90 ± 0.71*	12.30 ± 0.35
PCV (%)	35.40 ± 1.65	37.70 ± 2.10	39.00 ± 2.32*	37.00 ± 1.52
MCV (fL)	65.00 ± 0.68	64.00 ± 1.27	64.00 ± 1.20	66.40 ± 0.83
MCH (pg)	19.60 ± 0.32	18.80 ± 0.23	18.80 ± 0.38	19.80 ± 0.22
MCHC (g/dL)	32.70 ± 0.55	32.60 ± 0.57	33.10 ± 0.82	33.20 ± 0.41
WBC (×10^9/L)	20.50 ± 1.30	20.60 ± 1.82	18.10 ± 1.25	26.10 ±1.65*
PLT (×10^9/L)	610.00 ± 57.0	635.00 ± 42.8*	636.00 ± 49.5*	599.00 ± 42.9
LYMPH (%)	80.20 ± 1.55	78.30 ± 3.43	87.90 ± 1.84	80.00 ± 2.58
NEUT (%)	17.20 ± 1.79	18.70 ± 1.85	17.20 ± 1.48	17.40 ± 2.56
MXD (%)	2.60 ± 0.35	2.50 ± 0.75	2.03 ± 0.46	2.00 ± 0.48

RBC = Red Blood Cell, Hb = Haemoglobin, PCV = Packed Cell Volume, MCV = Mean Cell Volume, MCH = Mean Cell Concentration, MCHC = Mean Cell Haemoglobin Concentration, WBC = White Blood Cells count, PLT = Platelets, LYMPH = Lymphocytes, NEUT= Neutrophil, MXD = Monocytes.
**= Significantly different from control at p < 0.05*

Table 3. Effect of aqueous extract of *A. nilotica* root on total cholesterol in rats

Treatment (mg/kg b.wt)	Total cholesterol (mg/dL)	
	Male	Female
Control	73.50 ± 2.57	71.50 ± 2.35
125	71.20 ± 1.72	69.00 ± 1.94
250	70.00 ± 1.36	67.50 ± 2.76
500	69.30 ± 1.98*	66.20 ± 2.14*

*n= 6 ± SEM, * = significantly different from control at p < 0.05*

Table 4. Effect of aqueous extract of *A. nilotica* root on triacylglycerol concentration (mg/dl) in rats

Treatment (mg/kg b.wt)	Serum triacylglycerol concentration (mg/dL)	
	Male	Female
Control	57.80 ± 1.42	58.40 ± 1.64
125	55.80 ± 1.64	56.20 ± 1.86
250	54.20 ± 1.65	54.00 ± 1.85*
500	53.20 ± 1.58*	54.00 ± 1.70*

*n= 6 ± SEM, * = significantly different from control at p < 0.05*

3.5 Effect of *A. nilotica* on Serum High Density Lipoprotein-Cholesterol (HDL-C) and Low Density Lipoprotein-Cholesterol (LDL-C) Concentration

The extract did not produce significant effect on serum HDL and LDL concentration, in both male and female rats when compared to control (Tables 5 and 6).

Table 5. Effect of aqueous extract of *A. nilotica* root on serum HDL-cholesterol concentration in rats

Treatment (mg.kg b.wt)	Serum HDL-cholesterol concentration (mg/dL)	
	Male	Female
Control	46.60 ± 1.56	46.00 ± 1.34
125	45.80 ± 1.27	44.80 ± 1.15
250	46.20 ± 1.34	45.80 ± 1.12
500	46.80 ± 1.38	47.00 ± 1.16

n= 6 ± SEM

3.6 Effect of *A. nilotica* on Atherogenic Index

The extract did not produce any significant change in the atherogenic index (Total Cholesterol / HDL) of rats (Table 7).

Table 6. Effect of aqueous extract of *A. nilotica* root on serum LDL-Cholesterol concentration in rats

Treatment (mg/kg b.wt)	Serum LDL-cholesterol concentration (mg/dL)	
	Male	Female
Control	52.40 ± 1.86	51.00 ± 1.64
125	50.80 ± 1.48	48.40 ± 1.26
250	50.70 ± 1.49	51.10 ± 1.25
500	52.20 ± 1.41	51.80 ± 1.36

n= 6 ± SEM

Table 7. Effect of aqueous extract of *A. nilotica* root on atherogenic index in rats

Treatment (mg/kg b.wt)	Atherogenic index (Total Cholesterol/HDL)	
	Male	Female
Control	1.58 ± 0.19	1.55 ± 0.14
125	1.55 ± 0.16	1.54 ± 0.17
250	1.51 ± 0.13	1.47 ± 0.15
500	1.48 ± 0.12	1.32 ± 0.15

n= 6 ± SEM

4. DISCUSSION

Phytochemical analyses of aqueous extract of *A. nilotica* root revealed the presence of significant amount of secondary metabolites (Table 1) which have been reported to possess potent and appreciable medicinal potentials [2,10,17]. Their presence in extract of *A. nilotica* root might be responsible for some of the various pharmacological effects of the extract. Analysis of haematological parameters is a relevant part of risk assessment of medicinal plants, as the changes observed in the blood components may be indicative of possible human toxicity when data are extrapolated from animal studies to human [11]. The extract produced significant changes in some of the haematological parameters studied. The significant increase in the red blood cells, haemoglobin and packed cell volume (PCV) at the dose of 250 and 500 mg/kg b.w in the rats suggest that the extract could possess erythropoetic activity which will enhance the haemoglobin and PCV level and thereby correct anaemia commonly seen in malaria infection especially among children and pregnant women. This erythropoetic activity could be attributed to the significant level of iron in the extract [18]. Significantly high level of platelet counts in the treated groups when compared with control, suggest that the extract

may increase the risk of platelet aggregation and thromboembolism at this high dose (Table 2).

The increase in WBC count implies that the extract might possess immune-enhancing properties at high dose of 500 mg/kg b.w. Saponins content of the root extract might be responsible for the immune-enhancing property as it has been reported by Abdulelah and Zainal-Abidin, [19] to possess some immune-stimulating effects in rats.

Lipids are essential macronutrient and because they are relatively insoluble in aqueous media, they are transported in body fluids as soluble protein complexes called lipoproteins [8,20]. The significant decrease ($p < 0.05$) in concentration of serum cholesterol (Table 3) at the dose of 500 mg/kg body weight without a corresponding change in the serum HDL-C and LDL-C when compared to the control (Table 4), suggests that the extract might possess cholesterol-lowering effect and thus likely to reduce the risk of cardiovascular diseases. The serum cholesterol-lowering effect of the aqueous extract may be attributed to the presence of saponins identified as one of the phytochemicals in the root of this plant [7]. Plant saponins have been reported to inhibit cholesterol absorption from the intestinal lumen in experimental animals, and consequently reduce the concentration of serum cholesterol [21]. This may be due to the ability of saponins to complex with cholesterol in the digestive tract or a direct effect of plant saponins on cholesterol metabolism [21]. Reduction in serum cholesterol and triglycerides was also observed by Umar [22], after administration of aqueous pod extract of *Acacia nilotica* to albino rats. Similar reduction in serum cholesterol and triglyceride was observed with *Phyllantus reticulatus* stem and leaves [23], and *Piliostigma thonningii* leaves [24].

In addition, the atherogenic index (Table 7) at all the doses administered is not significantly different when compared to the control. Atherogenic index (Castelli index) is a measure of cardiovascular disease risk and highly predictive of cardiovascular disease [25,26]. High atherogenic index may be due to an increase in the atherogenic component contained in the numerator, a decrease in the anti-atherosclerotic trait of the denominator, or both [26]. This result is similar to the atherogenic index reported by Adesokan [27] following administration of aqueous extract of *Enantia chlorantha* to rats.

5. CONCLUSION

Aqueous root extract of *Acacia nilotica* significantly increased packed cell volume and reduced serum cholesterol level; therefore it could serve as a phytomedicine for treatment of anaemia and hypercholesterolemia.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures involving laboratory animals in this study complied with National Academy of Sciences guidelines on handling of experimental animals and ethical approval for this study was obtained from animal ethics committee of NIPRD.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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