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Evaluation of the Effect of Crude Extract and Aqueous Fraction of *Acacia nilotica* Leaves on Haematological Indices and Serum Electrolytes Levels on Diabetic Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author YT designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors RAI and AM managed the analyses of the study. Author EDE, AJ, KAM and AM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to evaluate the effect of crude and aqueous fraction of *Acacia nilotica* on diabetic Wistar Rats. Alloxan at a dose of 150 mg/kg body weight was administered to the rats intraperitoneally to induce diabetes. Thirty rats divided into six groups of five animals (n = 5) each were used for the experiment: Group 1 served as normal control group, Group 2 administered insulin (6 I.U/kg),Group 3 administered 500 mg/kg of crude extract. Group 4 administered 1000 mg/kg crude extract, Group 5 administered 500 mg/kg aqueous fraction and Group 6 received 1000 mg/kg aqueous fraction of *Acacia nilotica*. There was no significant change in the haematological indices, serum urea, creatinine and potassium compared to control. However, there was a significant increase ($p<0.05$) in the serum sodium and chloride levels when compared to control.

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The result indicated that the crude and aqueous fraction *of A. nilotica* has no effect on haematological indices, urea, creatinine, potassium. However, the fraction has effect on serum sodium and chloride levels on diabetic rats.

Keywords: Alloxan; crude extract; aqueous fraction; haematological indices.

1. INTRODUCTION

Diabetes is of the leading cause of morbidity and mortality in the world, with an estimated value of about 346 million adults were affected in the year 2011 [1]. The prevalence rate is expected to increase significantly between the years 2005 to 2030, with the highest increase expected in low- and middle-income developing countries of the African, Asian, and South American regions [1]. Diabetes is also associated with macro- and micro-vascular complications [2,3]. Therefore, there is also much larger burden in the form of lost productivity as a result of restricted daily activity in diabetic patients [3]. In diabetes, there are metabolic imbalance to nerve, blood vessel degeneration, anemia and electrolyte imbalance. Herbal medicine is still the mainstay of about 70-80% of world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with human body with lesser side effects. *Acacia nilotica* is a known plant used as herbal medicine [4]. It is used for treatment of different ailment traditionally. The Bark of the plant A. *nilotica* has been used for treatment of the following, haemorrhages, colds, diarrhoea, tuberculosis and leprosy while the roots have been used as an aphrodisiac in erectile dysfunction and the flowers for treatment of syphilis lesions [5]. The Zulus tribe in South Africa take a decoction of the bark as a cough remedy [5,6]. Other parts of the plant were used for the treatment of eye diseases, as a tranquillizer and as an aphrodisiac [7]. The plant leaves were used in the treatment of menstrual problems, eye infections, sores caused by leprosy, ulcers and indigestion [5]. The aim of this study was to evaluate of the effect of crude extract and aqueous fraction of *Acacia nilotica* leaves on haematological indices and serum electrolytes levels of diabetic Wistar Rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental animals

A total of 30 Wistar rats of both sexes between the age of 10 to 12 weeks and weighed between 120-150 gm 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, and fed on standard feeds (Vital feeds, Jos, Nigeria) with access to water *ad libitum*.

2.1.2 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, at the herbarium section of the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria, where a voucher specimen (No. 698) has been deposited for future reference.

2.1.3 Extraction of plant material

The leaves of *Acacia nilotica* were air dried under the shade and were ground into fine powder using mortar and pestle. Two hundred gm of the powdered material was macerated in 100% methanol at room temperature for 24 h and then filtered using Whatman filter paper 1. The filtrate was then evaporated to dryness in an oven at 37ºC to get the crude extract and kept in a sealed container at 4ºC in a refrigerator until use. Also 100 gm of the powdered material was macerated with distilled water at room temperature for 24 h. It was then filtered using Whatman filter paper No1. The filtrate was evaporated to dryness in an oven at 37ºC. A brownish residue weighing 85 gm was obtained and kept in a sealed container at 4ºC in a refrigerator until use.

2.1.4 Chemical and drug 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).

2.2 Method

2.2.1 Preliminary phytochemical screening

The fractions were subjected to preliminary phytochemical screening test for the presence of secondary metabolites according to the method described by Trease [8].

2.2.2 Acute toxicity studies (LD_{50})

The method previously described by Lorke [9] was adopted. Briefly, 13 mice were used for each extract. In the first phase, three doses of the methanol leaves extract (10, 100 and 1000 mg/kg) were administered to three groups each containing three mice. In the second phase, more specific doses were administered to four groups each containing one mouse. The median Lethal dose (LD_{50}) was determined as the geometric mean of the highest non-lethal dose and the lowest lethal dose of which there is 1/1 and 0/1survival. The same procedure was repeated for the aqueous fraction.

2.2.3 Induction of experimental 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). The animals were fasted for 16–18 h 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 [10]. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia [11]. The diabetes was assessed in alloxan-induced rats by determining the blood glucose concentration at 72 h after injection of alloxan. The rats with blood glucose level above 200 mg/dl were then selected for the study.

2.2.4 Experimental design

The alloxan -induced diabetic Wistar rats were randomly assigned into six groups (1-6) of five rats in each group.

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

2.2.5 Determination of blood glucose levels

Blood samples for blood glucose determination were collected from the tail and determination of the blood glucose level was done by glucose-oxidase method using the ONE TOUCH Basic (Lifescan, Milpitas, CA) instrument and results were reported as mg/dl [12].

2.2.6 Collection of blood and preparation of serum samples

After the last day of administration the animals were euthanized and blood samples were drawn from the heart of each by cardiac puncture into plain tubes with drops of Ethylenediaminetetraacetic acid (EDTA) for determination of haematological parameters and for the serum electrolytes assay, the blood were allowed to clot and the serum separated by centrifugation using Denley BS400centrifuge (England) at 3000 r p m for 15minutes and the serum collected. The sera was stored at - 4ºC for serum electrolytes analysis until use.

2.2.7 Determination of haematological parameters

Determination of haematological parameters such as haemoglobin (Hb), haematocrit (PCV), red cell count, total white blood cell count (TWBC) and its differentials was done using standard procedures described by Decie [13].

2.2.8 Determination of serum electrolytes

Serum sodium and potassium ions were measured by the flame photometry method of Vogel [14], and bicarbonate ions were determined using the titration method of Segal

[15]. Chloride ions were analyzed using the method of Schales [16]. Calcium and phosphate ions were determined according to laboratory procedures of Randox Laboratories Limited kits, United Kingdom.

2.2.9 Statistical analysis

Data obtained were expressed as mean ± SEM. The data were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. The values of $P<0.05$ were considered as significant [17].

3. RESULTS

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

3.1 Acute Toxicity Study (LD_{50})

After 12-18 h of administration of the crude and aqueous fraction, the following were noticed as sign of toxicity: decrease locomotor activity, decrease in sensitivity to touch decreased feed intake, and prostration. The median lethal dose (LD_{50}) in rats was 2,154 mg/kg body weight for crude and aqueous fraction.

3.2 Effect of Crude Extract and Aqueous Fraction of *Acacia nilotica* on Haematological Indices on Diabetic Wistar Rats

Table 1 below shows the effect of crude extract and aqueous fraction of *Acacia nilotica* on haematological indices on diabetic Wistar rats. There was no statistically significant change in blood parameters of diabetic rats treated with the plant as compared to control Table 1.

3.3 Effect of Crude Extract and Aqueous Fraction of *Acacia nilotica* on Serum Electrolytes Levels on Diabetic Wistar Rats

Table 2 the effect of crude and aqueous fraction of *Acacia nilotica* on serum electrolytes levels on diabetic Wistar rats. The result indicated that there was no significant change in the serum levels of urea, creatinine and potassium ions at the doses tested. However, there was a significant increase in the serum levels of sodium and chloride ions compared to control group. And there was a significant increase ($P < 0.05$) in the sodium and potassium levels at the doses of 500 and 1000 mg/kg of the crude extract, compared to control. As regard to the aqueous fraction, there was a significant increase in the chloride level at the dose of 1000 mg/kg, compared to control, while there was no significant change in the chloride level at the dose of 500 mg/kg, compared to control.

4. DISCUSSION

The present study evaluated the effect of crude extract and aqueous fraction of *Acacia nilotica* leaves on haematological indices and serum electrolytes levels on diabetic Wistar rats. The study of haematological parameters has been reported to be useful indices of evaluating the toxicity of plant extract in rodents [18]. This can not only be used to determine the extent of deleterious effects of plant extract on the blood of an animal, also it can also be used to explain blood related functions of a plant or secondary metabolites [19]. From our study, it indicated that there was no statistically significant change on the haematological parameters with different doses of crude and aqueous fraction of *Acacia nilotica*. This implied that, the non-significant change in white blood cell and differentials counts might indicate an increase in anti-inflammatory effect, which is not a boost in the immune system on diabetic Wistar rats. Other studies revealed that assessing the level of excretory metabolites such as urea, creatinine and electrolyte levels can be implored as a reliable tool to evaluate renal function [20,21]. There was no significant change in the serum urea, creatinine and potassium levels in the groups administered the crude extract at tested doses of 500 and 1000 mg/kg as well as the aqueous fraction at the tested doses of 500 and 1000 mg/kg of *Acacia nilotica,* compared to control. However, there was a significant increase ($P<0.05$) in the serum sodium and chloride levels in the groups administered 500 and 1000 mg/kg doses of the crude extract, compared to control. Serum sodium ion level was higher in the test rats studied. Hypernatremia is rare but does occur when there is loss of body fluids containing less sodium than plasma along with water intake restriction or if there is excessive sodium intake with limited liquid intake [22].

Table 1. Shows the effect of crude and aqueous fraction of *Acacia nilotica* on Haematological indices on diabetic wistar rats

Groups (n=5)	PCV (%)	Hb(g/dl)	WBC×10^9/L	RBC (10^{12}/L)	Neutrophils (%)	Eosinophils (%)	Monocytes (%)	Lymphocytes (%)
Control	38.50±1.56	12.82±0.44	4.73±0.26	6.35±0.36	19.50±1.04	1.25±0.25	2.50±0.28	76.75±1.03
Insulin (6.I.U/kg)	34.50± 2.10	11.50±0.82	4.95±0.56	5.95±0.53	16.00±1.68	2.25±0.25	4.75±1.49	78.50±1.93
Crude Extract (500 mg/kg)	41.00±1.35 ^{ns}	13.55±0.43 ^{ns}	4.28±0.65 ^{ns}	6.83±0.06 ^{ns}	17.75±2.81^{ns}	2.00±0.00 ^{ns}	3.50±0.50 ^{ns}	76.75±2.49 ^{ns}
Crude Extract (1000 mg/kg)	35.75±1.80 ^{ns}	12.13±0.68 ^{ns}	5.80±0.47 ^{ns}	5.55±0.36 ^{ns}	13.00±1.35 ^{ns}	2.50±0.29 ^{ns}	3.75±1.10 ^{ns}	80.75±1.10 ^{ns}
Aqueous Fraction (500 mg/kg)	34.50±1.85 ^{ns}	11.65±0.65	5.08±0.19 ^{ns}	6.30±0.19 ^{ns}	15.75±2.14 ^{ns}	1.75±0.48 ^{ns}	2.75±0.25 ^{ns}	79.75±2.05 ^{ns}
Aqueous Fraction (1000 mg/kg)	40.00 3.63^{ns}	13.10±1.22 ^{ns}	4.80±0.28 ^{ns}	6.78±0.65 ^{ns}	27.75±1.11 ^{ns}	2.25±0.25 ^{ns}	2.25±0.62 ^{ns}	67.75±1.31 ^{ns}

Values are expressed as mean ± SEM

Value considered statistically significant when compared with control group at p<0.05 and ns = not significant

Table 2. Effect of crude and aqueous fraction of *Acacia nilotica* on Serum electrolytes levels on diabetic wistar rats

Groups (n=5)	Urea (mmol/L)	Sodium(mmol/L)	Potassium(mmol/L)	Chloride (mmol/L)	Creatinine (mmol/L)
Control (Distilled Water)	21.83±1.26	134.23±2.10	4.88±0.17	93.53±1.51	1.13±0.17
Insulin (6.I.U/kg)	19.90±0.81	133.93±2.74	5.15±0.34	98.20±1.23	1.03±0.11
Methanol crude Extract (500 mg/kg)	23.35±1.04 ^{ns}	148.40±8.04 ^{a}	6.53±0.23 ^{ns}	112.20±5.60^{a}	2.13±0.15^{ns}
Methanol crude Extract (1000 mg/kg)	30.70±2.77 ^{ns}	158.10±6.82 ^{a}	7.85±0.28^{ns}	118.95±6.26^{a}	1.98±0.23^{ns}
Aqueous Fraction (500 mg/kg)	21.15±0.77 ^{ns}	152.10±3.21 ^{a}	6.98±0.48^{ns}	109.30±1.56^{ns}	1.58±0.14 ^{ns}
Aqueous Fraction (1000 mg/kg)	25.53±2.67 ^{ns}	160.30±7.45^{a}	7.48±0.85^{ns}	118.43±8.10^{a}	1.95±0.26^{ns}

Values are expressed as mean ± SEM

Value considered statistically significant when compared with control group at p<0.05 and ns = not significant

Also reported that hypernatremia always indicates water depletion [23] Water was not however restricted in this study thus, this increase is suspected to be due to the inability of the kidneys to excrete adequate sodium from the tubular fluid and the fraction may contain some sodium based compounds. These may have led to the excess sodium ion levels in the the study. In relation to the aqueous fraction there was a significant increase ($P<0.05$) in the chloride level at the dose of 1000 mg/kg when compared to control, while there was no significant change in the chloride level at the dose of 500 mg/kg when compared to control. Excretion of glucose in urine in diabetic patient usually leads to osmotic diuresis leading to loss of electrolytes especially sodium ion [24]. This significant increase in serum electrolytes levels such as sodium might be due to peripheral tissue glucose absorption through sodium glucose co transport. On the other hand changes in serum potassium level usually alternate with those of sodium [24]. Diabetic acidosis which is more pronounced in diabetic conditions which causes reduction in potassium excretion in urine, and therefore, leading to increase in retention. In our study, the serum in potassium did not significantly change, compared to control. Type 1 diabetes mellitus, there is deficiency of insulin leads to inability of glucose to reach the extra hepatic tissues [24].

5. CONCLUSION

Finally, *A. nilotica* showed no significant change on haematological parameters as well as serum urea, creatinine and potassium levels. However, showed a significant increase in serum sodium and chloride levels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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