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Effects of Ethylacetate and n-Butanol Fractions of *Acacia nilotica* Extract on Some Haematological Profile and Serum Electrolytes Levels of Alloxan-induced Diabetic Wistar Rats

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Abstract
The aim of this study was to examine the effects of ethylacetate and n-butanol fraction of *Acacia nilotica* fractions on some haematological parameters and serum electrolytes levels of alloxan-induced diabetic wistar rats. Thirty (30) Wistar rats were used in this study. Group 1, administered distilled water, served as negative control; Group 2: administered insulin 6.iu/kg, served as positive control; Group 3: administered 50mg/kg *b.w* of ethyl acetate fraction; Group 4: administered 100mg/kg *b.w* of ethyl acetate fraction; Group 5: administered 100mg/kg *b.w* of n-butanol fraction and Group 6: Administered 200mg/kg body weight of n-butanol fraction. All regiments were given intraperitoneally once daily to the animals for a period of two weeks .At the end of the two weeks, the animals were sacrificed and the blood samples were collected for biochemical assays. The results obtained showed that there was no statistically significant change $(P>0.05)$ in the packed cell volume, red cell count, haemoglobin concentration and as well as white blood cells and its differential counts in groups treated with all doses of ethyl acetate and n-butanol fractions of *Acacia nilotica* in the experimental animals when compared with the diabetic control untreated group. There was significantly increase $(p<0.05)$ urea level, potassium and chloride ions concentrations in the group treated with 100 mg/kg b w ethyl acetate. As regards to the 200 mg/kg of the n-butanol fraction there was a significant increase in the level of urea when compared with the control untreated group.

Keywords: *Acacia nilotica,* Red cell count, Haemoglobin concentration, White blood cell count, Potassium ion, Sodium ion

1. Introduction
Diabetes mellitus is a chronic, widely spread human disease. It is a group of chronic metabolic disorders characterized by chronic hyperglycaemia as a result of relative or absolute lack of insulin or the actions of insulin [1]. Chronic hyperglycaemia during diabetes increase non enzymatic glycation of proteins that later leads to secondary complication. These complications include hypoglycaemia, diabetic ketoacidosis, non-ketotic syndrome, thirst, polyuria, visual blurriness, weight loss, hypertension, neuropathy, nephropathy and retinopathy [2]. *Acacia nilotica* is a thorny wattle native to India, Pakistan, Middle East and much of Africa. It is also called thorn mimosa or prickly acacia in Australia, lekkerruikpeul or scented thorn in South Africa. The species name nilotica was given by Linnaeus for these trees were best-known range along the Nile River [3]. *Acacia nilotica* is a tree 5–20 m high with a dense spherical crown, stems and branches usually dark to black colored, fissured bark, grey-pinkish slash, exuding a reddish low quality gum. The tree has thin, straight, light, grey spines in its axillaries pairs, usually in 3 to 12 pairs, 5 to 7.5 cm (3 in) long in young trees, mature trees commonly without thorns. The leaves are bipinnate, with 3-6 pairs of pinnulae and 10-30 pairs of leaflets each. Pods are strongly constricted, hairy, white-grey, thick and softly tormentors. Its seeds number approximately 8000/kg. The leaf stalks are heavy. Very small glands, almost un noticeable with the naked eye, can be found at the base of most of the upper pinnate pairs [4]. The present study examined the effects of ethylacetate and n-butanol fraction of *Acacia nilotica* fractions on some hematological parameters and serum electrolytes in alloxan-induced diabetic wistar rats.

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2. Materials and Methods
2.1 Materials
2.1.1 Chemicals and Drugs Used
All chemicals and drugs used were of analytical grades. Alloxan was purchased from Sigma chemicals (St Louis U.S.A) while Insulin and digital glucometer (Accu-chek Advantage) were obtained from pharmaceutical store in Zaria, Kaduna state.

2.1.2 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*.

2.1.3 Preparation of Fractions
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 30% distilled water and 70% ethanol 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 30% distilled water and 70% ethanol 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.

2.2 Method
2.2.1 Preliminary Phytochemical Screening
The extract was subjected to preliminary phytochemical screening test for the presence of secondary metabolites according to the method described by [5].

2.2.2 Acute Toxicity Studies (LD$_{50}$)
The LD$_{50}$ determination for each of the fractions was conducted separately using modified method of [6]. 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 intraperitoneally (ip) respectively. A fourth group received Tween-20 served as control. The rats were observed for clinical signs and symptoms of toxicity within 24 hours and death within 72 hours. Based on the results of phase one for the Ethylacetate extract, fifteen fresh rats with three per group were each treated with 600, 1000,1600 and 2900 mg extract/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 140, 225,370 and 600 mg extract/kg (ip) respectively. A fifth group served as control. Clinical signs and symptoms of toxic effects and mortality were then observed

for seven days. The LD$_{50}$ 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.

2.2.3 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 [7]. 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 6h. The rats were then kept for the next 24h on 5 % glucose solution bottles in their cages to prevent hypoglycemia [8]. 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.

2.2.4 Experimental Design
After the induction of diabetes, the alloxan induced diabetic Wistar rats were randomly assigned into the following groupings:
Group 1: Administered distilled water 1mg/kg *b.w* (*i,p*) and served as negative control
Group 2: Received standard dose of insulin 6.i.u/kg *b.w* (*i,p*) and served as positive control
Group 3: Received 50mg/kg *b.w* (*i,p*) of ethyl acetate fraction.
Group 4: Received 100mg/kg *b.w* (*i,p*) of ethylacetate fraction.
Group 5: Received 100mg/kg *b.w* (*i,p*) of n-butanol fraction.
Group 6: Received 200mg/kg *b.w* (*i,p*) of n-butanol fraction.

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 the glucose-oxidase principle (Beach and Turner, 1958) using the ONE TOUCH Basic (Lifescan, Milpitas, CA) instrument and results were reported as mg/dl [9].

2.2.6 Collection of Blood and Preparation of Serum Samples
After two weeks of treatment with fractions, blood samples were obtained from all animals in each group through cardiac puncture and placed in labeled sample bottles with drops of Ethylenediaminetetraacetic acid (EDTA) for determination of haematological parameters. For evaluation of serum electrolytes, blood sample from each animal was collected into plain tubes and allowed to clot and centrifuged at $1,957 \times g$ for 10 minutes. The sera was separated and stored at -4 ^{0}C for serum electrolytes analysis.

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 operative procedures according to [10].

2.2.8 Determination of Serum Electrolytes
Serum sodium and potassium ions were measured by the flame photometry method of [11], and bicarbonate ion was determined

using the titration method of [12]. Chloride ion was analyzed using the method of [13]. 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 obtained 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. All statistical analysis was evaluated using SPSS Version 17.0 software. The values of P<0.05 were considered as significant.

3. Results
3.1 Preliminary Phytochemical Screening
Preliminary phytochemical screenig of the two fractions of *Acacia nilotica* extracts revealed the presence of saponin, flavonoid, tannin and alkaloid.

3.2 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 8-12 hours of fractions administration. Early deaths were recorded after 12 hours and late deaths 48hours after fractions administration. The LD_{50} 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 ethylacetate fraction, there was 0% mortality at 1000mg/Kg and 33.3% mortality was the next

highest lethal dose at 1600mg/Kg. The LD_{50} of the Ethylacetate fraction was thus; $\sqrt{1000 \times 1600} = 1264.9$ mg/Kg. For the n-butanol fraction, there was 0% mortality at 370mg/Kg and 33.3% mortality was the next highest lethal dose at 600mg/Kg. The LD_{50} of the n-butanol fraction was thus; The LD_{50} was thus; $\sqrt{370 \times 600} = 471.2$ mg/Kg.

Effects of Ethyl Acetate and N-Butanol Fractions of *Acacia nilotica* on Haematological Indices in alloxan-induced Diabetic Wistar rats.
Table 1 shows the effects of ethyl acetate and n-butanol fractions of *Acacia nilotica* on haematological indices in alloxan-induced diabetic Wistar rats. The results obtained showed that there was no statistical significant change in the packed cell volume, red cell count, haemoglobin levels as well, as white blood cells and differential counts in all the groups treated with all doses of the fractions(ethyl acetate and n-butanol) when compared with the diabetic control untreated group.

Effects of ethyl acetate and n-butanol fractions of *Acacia nilotica* on serum electrolytes in alloxan-induced diabetic Wistar rats.
Table 2 shows the effects of ethyl acetate and n-butanol fractions of *Acacia nilotica* on serum electrolytes in alloxan-induced diabetic Wistar rats. The results obtained revealed a significant increase (P < 0.05) urea level, potassium and chloride ions concentrations in the group treated with 100 mg/kg b w ethyl acetate fraction of the plant, while there was a significantly elevated (P < 0.05) level only in urea level in the group that were administered 200 mg/kg b w n-butanol fractions respectively when compared with the diabetic control untreated group

Table 1: Effect of ethyl acetate and n-butanol fractions of *Acacia nilotica* on some haematological indices in Alloxan-induced diabetic Wistar Rats

Groups and Treatment Given (n=5)	PCV (%)	Hb (g/dl)	WBC $\times 10^9$/L	RBC (10^{12}/L)	Neutrophils (%)	Eosinophils (%)	Monocytes (%)	Lymphocytes (%)
Control	38.50± 1.55	12.82 ± 0.44	4.73 ± 0.26	6.35 ± 0.36	19.50 ± 1.04	1.25 ± 0.29	2.50 ± 0.29	36.50 ± 0.87
Insulin (6.iu/kg)	34.50 ± 2.10 ns	11.50± 0.82 ns	4.95 ± 0.56 ns	5.95 ± 0.53 ns	16.00± 1.68 ns	2.00 ± 0.41	3.50 ± 1.49	78.50 ± 1.94 a
Ethyl Acetate (50mg/kg)	37.67± 0.88 ns	12.30± 0.42 ns	4.67 ± 0.26 ns	5.57 ± 0.30 ns	22.67± 2.33a	1.00 ± 0.00 ns	3.33± 0.88 ns	73.00 ± 2.00 ns
Ethyl Acetate (100mg/kg)	39.25 ± 1.11 ns	13.13 ± 0.38 ns	5.13± 0.42 ns	5.05 ± 0.59 ns	15.50± 1.66 ns	1.25 ± 0.48 ns	3.25 ± 1.48 ns	80.75± 1.35 ns
n-butanol (100mg/kg)	37.0 ± 3.29 ns	33.0 ± 20.01 ns	4.68± 0.41 ns	6.25± 0.69 ns	15.00 ± 1.22 ns	1.50 ± .29 ns	3.75± 0.25 ns	79.75 ± 0.85 ns
n-butanol (200mg/kg)	8.25± 1.31ns	12.93 ± 0.54 ns	4.53 ± 0.56 ns	5.35 ± 0.41 ns	17.00 ± 2.94 ns	1.50 ± 0.29 ns	3.00 ± 0.41 ns	78.25 ± 2.56 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 Ethyl Acetate and n-butanol fractions of *Acacia nilotica* on serum electrolyte levels in Alloxan-induced diabetic Wistar Rats

Groups and Treatment Given (n=5)	Urea (mmol/L)	Sodium(mmol/L)	Potassium(mmol/L)	Chloride (mmol/L)	Creatinine (mmol/L)
Control	21.83 ± 1.26	134.23 ± 2.10	4.88 ± 0.17	93.68 ± 1.39	1.13 ± 0.17
Insulin (6.iu/kg)	19.90 ± 0.81 ns	133.93 ± 2.74 ns	5.15 ± 0.34 ns	98.20 ± 1.23 ns	1.03 ± 0.11 ns
Ethyl acetate (50 mg/kg)	24.90 ± 2.39 ns	138.85 ± 1.59 ns	5.43 ± 0.34 ns	98.98 ± 3.28ns	1.23 ± 0.21ns
Ethylacetate (100mg/kg)	30.30 ± 1.39a	132.63 ± 7.68 ns	6.60 ± 0.32a	102.93 ± 3.24a	1.40 ± 0.28ns
n- butanol (100mg/kg)	20.80 ± 1.06 ns	141.13 ± 2.25 ns	5.85 ± 0.33ns	94.80 ± 1.56ns	0.95 ± 0.16 ns
n butanol (200mg/kg)	26.95 ± 2.09 a	133.93 ± 2.74ns	5.15 ± 0.34ns	98.20 ± 1.23ns	1.03 ± 0.11ns

Values are expressed as mean ± SEM
Value considered statistically significant when compared with control group at P < 0.05 and ns = not significant

4. Discussion

The present research work examined the effects of ethylacetate and n-butanol fraction of *Acacia nilotica* fractions on some hematological parameters and serum electrolytes in alloxan-induced diabetic Wistar rats. The kidney plays an important role in the excretion and regulation of osmolytes, especially in diabetes mellitus [14]. Thus, any damage to the kidney will exacerbate diabetes mellitus. Available evidence indicates that the kidney is a major target of oxidative stress in diabetes mellitus [14-15]. The results obtained revealed a significant ($P < 0.05$) increase in the urea and creatinine levels in the group administered 100 mg/kg ethylacetate fraction when compared with the control untreated group. Serum creatinine and urea are well-established markers of glomerular filtration rate (GFR) (Sirwal *et al.*, 2004). Increased catabolism of proteins coupled with the diminished ability to excrete the nitrogenous waste might have accounted for the raised urea and creatinine in serum of diabetic patients. The action of the combination may be attributed to its protective effect through the inhibition of the formation of Reactive oxygen species.

But there was no significant change in the group administered 50 mg/kg ethylacetate fraction. In relation to the n-butanol fraction at the tested doses (100 and 200 mg/kg) there was a significant increase in the urea level in the group administered 200 mg/kg n-butanol fraction when compared with the control untreated group, while there was no significant change with the 100 mg/kg . As regards to the potassium and chloride ions levels the group administered 100 mg/kg ethylacetate, there was a significant increase ($P<0.05$) when compared with control untreated. 100 mg/kg n- butanol fraction did not produce significant change when compared with the control untreated group.
The reference drug (insulin 6.i.u/kg) produced no significant change in all the tested profiles when compared with the control untreated group.

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds toxins, chemicals and plant extracts on the blood constituents of animals. Also in this study, the effects of ethylacetate and n-butanol fraction of *Acacia nilotica* fractions on some haematological parameters such as packed cell volume, haemaglobin concentration, red cells count, white blood cells count and its differential count in alloxan-induced diabetic animals were assessed. The results obtained showed that there was no statistically significant change in the packed cell volume, red cells count, haemoglobin level, as well as white blood cells and its differential counts in groups treated with different doses of ethyl acetate and n-butanol fraction of *Acacia nilotica* in the experimental animals when compared with the diabetic untreated control group.

5. Conclusion

Therefore, it can be concluded that administration of ethylacetate and the n-butanol fractions of *Acacia nilotica* significant increase urea , potassium and chloride levels in the group treated with 100 mg/kg b w ethyl acetate. While there was a significant increase level in urea level in the group administered 200 mg/kg *b w* n-butanol fraction.

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