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Haematological Profile of Rats Administered Aqueous Extract of *Canavalia ensiformis* (DC) Seed

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Abstract
In Northern Ibie, a settlement in Auchi, Edo State, water extract of Canavalia ensiformis (DC) seed has found use in the treatment of diabetes mellitus. This study investigates the haematological profile, acute toxicity and body mass changes of normal Wistar strain albino rats administered daily oral graded doses of an aqueous seed extract for a 14-day period using standardized procedures. No mortalities were recorded, suggesting relative safety up to the 600 mg/kg body weight dose. Slight non-significant decreases ($p>0.05$) observed in haemoglobin (Hb) concentration, total erythrocyte (RBC) count and packed cell volume (PCV) were not dose-dependent compared to normal rats; thus indicating the extract did not cause any adverse effects on the haematopoietic system of the animals at dosages 100, 200 and 400 mg/kg body weight. However, there was a 20.62% increase in leukocyte count (WBC) at the 600 mg/kg body weight dose, indicative of inflammation or toxicity and a 19.86% decrease in Hb, a 21.28% decrease in RBC and a 20.46% decrease in PCV. Thus caution at high dosing is recommended. No significant alterations ($p>0.05$) were observed with mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of all treated rats compared to control animals. Anthropometric data is suggestive of adequate composition of nutritive factors in Canavalia ensiformis seeds since all treated rats gained weight in a dose dependent manner. Overall, results from the preset study clearly indicate that, the aqueous extract of Canavalia ensiformis is best administered at the 400 mg/kg body weight dose for nutritional and health benefits. In addition, Canavalia ensiformis at 400 mg/kg body weight dose and above may elicit a sedative or tranquilizing effect.

Keywords: *Canavalia ensformis*, haematology, body mass, toxicity, albino rats

Introduction
Canavalia ensiformis DC (Family: Leguminosae) with the following common names jackbean (English), ikpakpa (Auchi), ikpakpa no kua (Bini), cat eye, horse bean, one eye bean and overlook (West Indies) is a climber usually growing on support. Though a native of Central America and West Indies, it has been widely cultivated in the humid tropics of Africa and Asia. The seeds though edible, have a bitter taste and so are boiled three times (with two changes of water) within two days before being eaten.

Scientific investigation has revealed the presence of growth inhibiting proteins canavalin, concanavalin A and B, the enzyme urease and the amino acid canavanine [1, 2] which must be detoxified before they are edible[3].

In Northern Ibie, a settlement in the Auchi Local Government Area of Edo State, the seed of Jackbean (*Canavalia ensiformis*) known locally as "Ikpakpa" is used for the treatment of diabetic persons. The seeds are boiled and the liquid is orally administered in no precise dosage to patients within 24 hours. They claim that the extract becomes toxic thereafter. Laboratory findings confirm the ethnomedicinal usage of the seeds of *Canavalia ensiformis* in treating diabetes mellitus[4]. This study investigates haematological parameters, acute toxicity and body mass changes in normal laboratory rats administered graded oral daily doses of the aqueous extract of *Canavalia ensiformis* seed for 14 days as part of a toxicological assessment profile of the seeds.

Materials and Methods
Canavalia ensiformis seeds were purchased from Auchi main market, Auchi, Edo State, Nigeria and subsequently identified at the Department of Plant Biology and Biotechnology Herbarium of the University of Benin, Benin City, Nigeria. The aqueous seed extract was prepared as described by Nimenibo-Uadia and Osagie[4].

Experimental Animals
Wistar strain albino rats weighing between 100 – 175 g were purchased from the University of Lagos Teaching Hospital (LUTH) Animal Unit, Lagos. The rats were acclimatized to laboratory conditions for two weeks and allowed access to fresh water and feed ad libitum (Bendel Feed and Flour Mill, Ewu, Nigeria). Animals were handled in accordance with the principles of Laboratory Animal Care (NIH Publication 85 – 93, revised 1985).

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Experimental Design

The rats were randomly divided into four groups of four rats each. By means of a gavage rats were administered the following doses orally, once a day for 14 consecutive days.

Group A: Normal rats received distilled water only (Control)
Group B: Normal rats received 100mg/kg body weight *C. ensiformis* aqueous extract
Group C: Normal rats received 200mg/kg body weight *C. ensiformis* aqueous extract
Group D: Normal rats received 400mg/kg body weight *C. ensiformis* aqueous extract
Group E: Normal rats received 600mg/kg body weight *C. ensiformis* aqueous extract

Body Mass Measurement

At the beginning of the experiment (Day 0) all the rats were weighed, and seven days later they were re-weighed (Day 8) and also at the end of the experimental period (Day 15).

Acute Toxicity Studies

The possible toxic effect of the extract was studied using the method of Wadood *et al.* [(5)]. Animals were observed for 8 h after oral administration of the extract to check for unusual behaviour and mortalities. They were further kept under observation for 7 days and closely monitored for signs of restlessness, excitement, intoxication and behavioural changes.

Blood Collection

Blood was collected by tail vein puncture from conscious rats at Day 0, 8 and 15. Approximately 2 ml of whole blood was collected into EDTA treated tubes and assessed for haematological parameters within 24 h.

Haematological Parameters

Determination of haematological parameters was adapted from Bernadette[(6)]. Haemoglobin (Hb) concentration was estimated using the cyanomethaemoglobin method. Packed cell volume (PCV) was determined using a microhaematocrit reading device. Total white blood cells (WBC) and red blood cells (RBC) were estimated using the improved Neubauer counting chamber. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated by formulae.

Statistical Analysis

Data are presented as means ± SEM of three determinations. The differences between the means of test and control groups were analysed by student's T-test. Statistical significance was set at $p \leq 0.05$.

Results

The results of the effects of graded oral doses of aqueous seed extract of *C. ensiformis* on body mass and haemotological parameters in normal Wistar rats are as depicted in Table 1 and Figures 1 to 7.

Body Mass Changes

Compared to the control animals (Group A), body weight increased in a dose-dependent manner (Table 1).

Table 1: Body mass (grams) of animals on Day 0, 8 and 15 following daily oral administration of aqueous seed extract of *Canavalia ensiformis*

Dose	**Day 0 Pre-Treatment (g)**	**Day 8 Post-Treatment (g)**	**Day 15 Post-Treatment (g)**	**Percentage Body Mass Difference from Day 0 (%)**
Control	164.50 ± 25.00	176.00 ± 24.65	180.00 ± 25.22	9.42
100 mg/kg	149.30 ± 7.62	167.00 ± 3.30	170.00 ± 3.00	13.87
200 mg/kg	133.00 ± 8.10	148.30 ± 4.28	154.00 ± 2.49	15.79
400 mg/kg	146.60 ± 16.98	172.60 ± 21.30	175.60 ± 16.63	19.78
600 mg/kg	154.60 ± 12.39	169.00 ± 12.30	185.60 ± 7.97	20.05

Values are means ± SEM of three determinations; number of rats per group = 4.

Acute Toxicity Studies

Administration of the aqueous seed extract of *C. ensiformis* did not elicit any observable toxicity and no mortalities were recorded. However, rats on the 400 mg/kg body weight and 600 mg/kg body weight dose seemed calmer with a fine coat of furs.

Haematological Indices

Following the 14-day oral treatment of normal rats with aqueous seed extract of *C. ensiformis*, the haemoglobin content (Fig. 1), RBC (Fig. 2), PCV (Fig. 3) and WBC (Fig. 4) dropped at the 100 mg/kg body weight dose. Slight increases were then observed as the dosage increased with the animals on the 200 mg/kg body weight dose recording the highest increases, though they were not statistically significant ($p>0.05$). Furthermore, the animals on the 600 mg/kg body weight recorded a decrease in haemoglobin content of 19.86 %, a 21.28 % decrease in RBC content, a 20.46 % decrease in PCV but a 20.62 % increase in WBC content after 14 days of treatment (Figures 1 to 4).

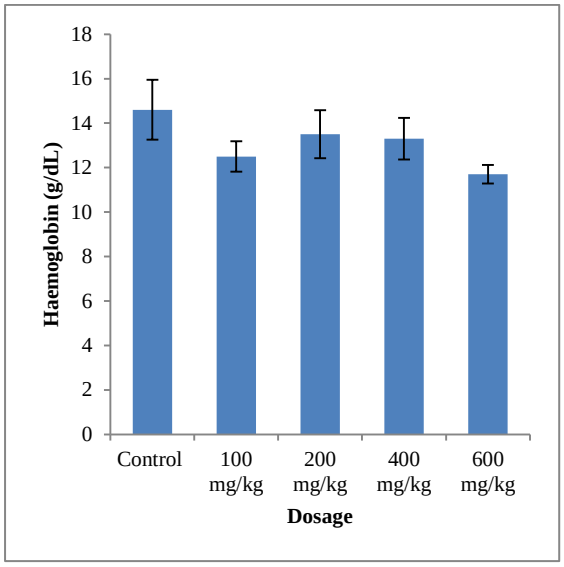

Fig. 2: Effect of aqueous seed extract of *C. ensiformis* on Haemogblobin concentration

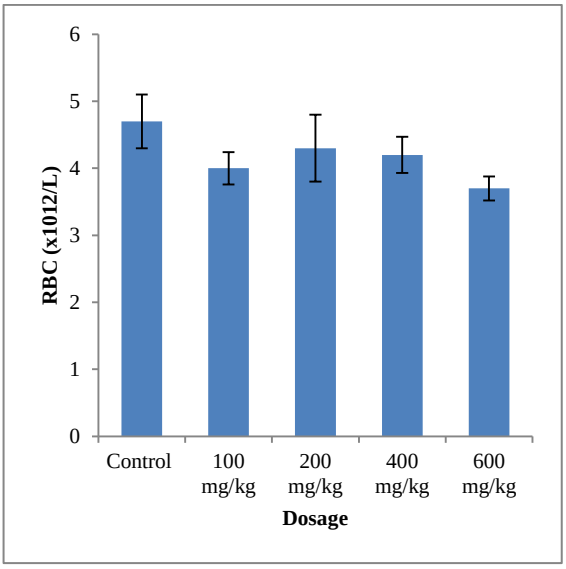

Fig. 1: Effect of aqueous seed extract of *C. ensiformis* on RBC Count

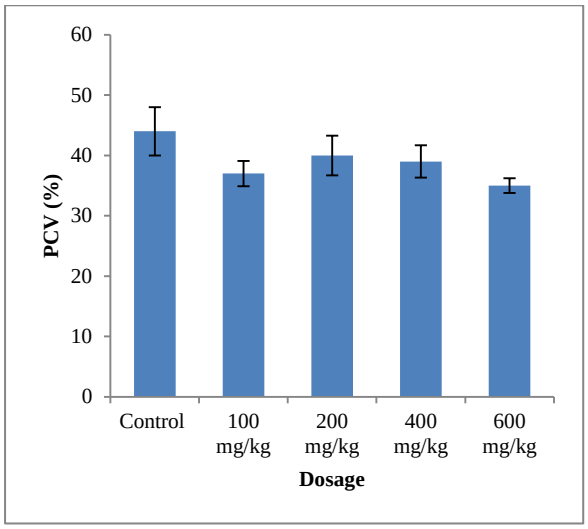

Fig. 3: Effect of aqueous seed extract of *C. ensiformis* on PCV

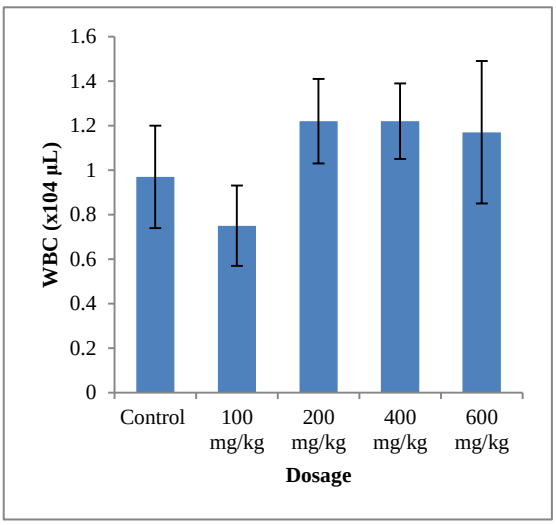

Fig. 4: Effect of aqueous seed extract of *C. ensiformis* on WBC Count

No statistically significant changes ($p>0.05$) were observed in MCV, MCH or MCHC of any of the rats on the various doses, compared to control rats (Figures 5 to 7) following 14 days of daily oral treatment with the extract.

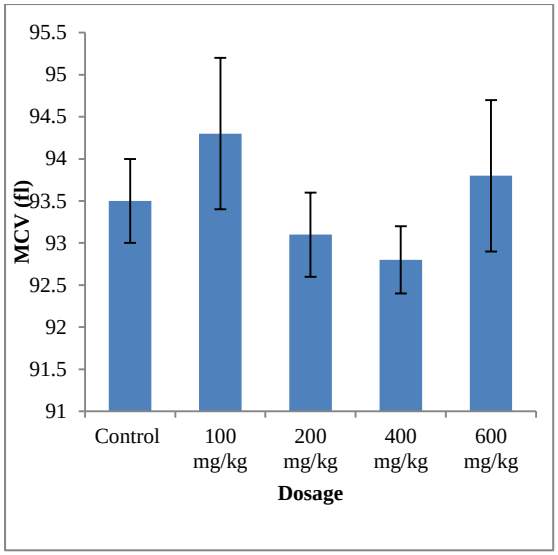

Fig. 5: Effect of aqueous seed extract of *C. ensiformis* on MCV

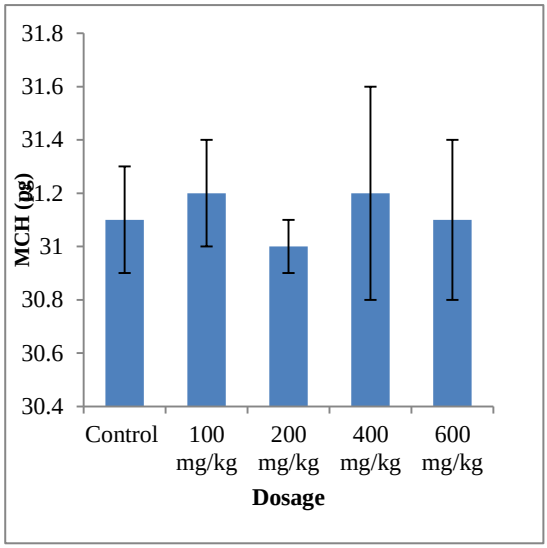

Fig. 6: Effect of aqueous seed extract of *C. ensiformis* on MCH

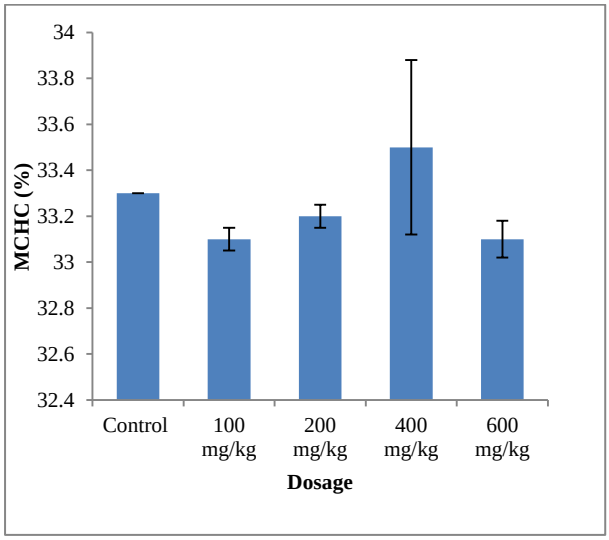

Fig. 7: Effect of aqueous seed extract of *C. ensiformis* on MCHC

Discussion

This study was designed to evaluate effects of administration of aqueous seed extract of *Canavalia ensiformis* DC (a medicinal plant) on haematological parameters of Wistar rats, as part of the toxicological evaluation of the bean seed. A blanket assumption should not be made about the safety of medicinal plants and it is necessary to ensure a thorough and detailed pharmacological and toxicological assessment of these plants and their products before approving them for therapeutic purpose. Seemingly innocuous plants may turn out to be toxic[7].

The increase in body weight in all the groups treated with the extract suggests the bean extract has enough nutritional factors to sustain life, since the animals were able to move, feed and metabolise their food components for conversion into the energy coin adenosine triphosphate (ATP). Body mass measurement is one of several anthropometric methods of assessing nutritional status in individuals or communities. While some researchers have reported increases in body weight, others have reported outright decreases depending on the medicinal plant being used [8].

Acute toxicity tests are critical since they give a rough idea about the nature of the candidate medicinal plant in addition to determining safe levels for clinical use[7]. Since no mortalities were recorded at the doses studied, it

indicates safety up to the 600 mg/kg body weight dose investigated in the present study. Further confirming the nutritive value of the beans, was the fine coat of furs observed on the animals on the 400 and 600 mg/kg body weight doses. The rats were also calmer and easier to handle compared to controls, suggesting *C. ensiformis* aqueous extract at high doses of 400 mg/kg and above may have sedative or tranquilizing effects.

Examination of the blood in haematology is performed because of the importance of determining the presence of anaemia and leukocyte changes [(9)].

The primary function of haemoglobin (Hb) within the erythrocyte is the carrying of oxygen and carbon dioxide to and from the tissues. The cyanomethaemoglobin method for Hb determination which we used in the present study is the reference method. A low level of Hb is a sign of anaemia and iron deficiency anaemia shows up as a low RBC count[(10)] which was not the case in our investigation since the decreases observed at the 600 mg/kg body weight dose were not statistically significant. While Zaoui *et al.*[(8)] have reported significant increases in haematocrit, RBC and Hb concentration in rats treated with *Nigella sativa* seed fixed oil, other workers have reported significant decrease in Hb concentration after administration of *Ocimum gratissimum*[(11)].

Determination of the number of leukocytes, erythrocytes and platelets in the blood has long been a fundamental procedure in haematology[(9)]. The leukocyte (WBC) count of rats on the 100, 200 and 400 mg/kg body weight doses did not vary significantly from controls in the present study. However, there was a 20.62% increase in WBC content following 14 days of treatment compared with the value for control rats. Leukocytosis is often defined as an elevated WBC count greater than 11.0 x 10^9 per L in non-pregnant adults caused by certain medications and chronic inflammatory conditions among others[(12)]. Thus, there may be toxic compounds or metabolites in the bean seed under study, whose concentration(s) may be injurious at high doses, suggesting a cautions use of the seed. Further studies are needed to ascertain this. In contrast to our study, Jimoh *et al.*[(11)] reported decreases in WBC count of rats treated with *Ocimum gratissimum* aqueous leaf extract.

The MCV, MCH and MCHC are RBC indices normally calculated to determine the size and Hb content of the average RBC. Furthermore, they serve as a quality control check used in differentiating anaemias[(6)]. Compared with control animals on distilled water, the MCV, MCH and MCHC of rats did not alter significantly on oral administration of the extract for 14 days, thus confirming the absence of anaemia. The normal MCV range of 80 – 100 fl is well within the reach of those recorded in the present study (92.80 – 94.6 fl). Values lower than 80 fl would have indicated iron deficiency anaemia, thalassemia or other conditions of defective iron utilization, liver disease or hypothyroidism[(6)]. The MCH range of 31.0 – 31.6 recorded for rats in this study falls well within the reference range for adults of 28 – 32 pg, even though, the MCH is not generally considered in the classification of anaemias. The MCHC values for the rats were in the reference range of 32 – 37 g/dl for normochromic cells[(6)].

Conclusion

Daily oral administration of *Canavalia ensiformis* (DC) aqueous seed extract for 14 days did not elicit any adverse effects on the haematopoietic system of Wistar rats at doses 100 – 400 mg/kg body weight. Nonetheless, a substantial increase (20.62%) in leukocytes (WBC) was observed at the 600 mg/kg body weight dose which portends inflammation or toxicity. This result and those from anthropometric and acute toxicity studies indicate the best dose may be at the 400 mg/kg body weight. In addition, *Canavalia ensiformis* seed extract appears to have a sedative or tranquilizing effect at doses 400 mg/kg body weight and above. Sub-chronic and chronic toxicity dosing studies are advocated.

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