

Screening for Toxic and Haematological Effect of Ethanol Extract of *Azadirachta Indica* in Wistar Rats Using Some Serum Enzymes as Markers

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Abstract

The leaves of *Azadirachta indica* plant was screened for haematological, toxic and serum enzyme activities in rats. Twenty rats were used and were grouped into 4 of 5 rats each. Group 1 was the negative control group administered distilled water. Groups 2, 3, and 4 were the treatment groups received 200, 400 and 800 mg/kg body weight of the *A. indica* extract respectively. The rats were dosed for 14 days, thereafter were sacrificed and blood collected by cardiac puncture for analysis. The effect of *A. indica* extract was checked on haematological parameters and serum enzymes activities. All results in treatment groups were compared with the normal control at statistical confidence of 95% ($p < 0.05$). There was progressive reduction of haematological parameters as the dose of the extract increased from 200, 400, to 800 mg/kg body weight. Haematological parameters, PCV, RBC, Haemoglobin showed decrease value which was not statistically significant at ($p < 0.05$). Total leukocyte count, showed progressively elevation by the extract though not statistically significant. Differential leukocyte count indicated very mild lymphocytosis neutropeania, monocytopenia and eosinopenia which were not statistically significant. Clinical biochemical parameters, *A. indica* extract demonstrated normal levels of the serum enzymes (AST, ALT and ALP) though there was slight decrease in a dose dependent fashion. Total protein was within normal range. The normal MCV, MCH and MCHC values suggests normocytic normochromic anaemic condition. The extract of *A. indica* is safe to blood cells, liver and kidney marker enzymes at dose < 800 mg/kg body weight.

Keywords: *Azadirachta indica*, Toxicity, Serum enzyme, Haematology.

الكشف عن التغيرات السمية والدمية لمستخلص الإيثانول لنبات *Azadirachta Indica* في الجرذان باستخدام بعض أنزيمات المصل كمؤشرات

الخلاصة

تم فحص أوراق نبات *Azadirachta indica* للكشف عن أنشطة إنزيم مصل الدم في الفئران. تم استخدام عشرين جردي وتم تقسيمها الى مجاميع 4 - 5 فئران لكل منها. كانت المجموعة 1 مجموعة السيطرة السلبية وأعطت الماء المقطر. بينما كانت المجموعات 2 و 3 و 4 هي مجموعات المعاملة حيث جرعت 200 و 400 و 800 ملغم / كغم من وزن الجسم لمستخلص *A. indica* على التوالي. تم تجريعها لمدة 14 يوماً ، تم قتلها وجمع الدم عن طريق ثقب القلب لاجراء التحليلات الكيمحيوية. تم قياس تأثير مستخلص *A. indica* على المعلمات الدموية وأنشطة إنزيمات المصل. تمت مقارنة جميع النتائج لمجموعات المعاملة مع السيطرة تحت مستوى المعنوية 95% كان هناك انخفاض تدريجي في المعلمات الدموية حيث زادت جرعة المستخلص من 200 ، 400 ، إلى 800 ملغم / كغم من وزن الجسم. أظهرت المعلمات الدموية ، PCV ، RBC ، الهيموجلوبين انخفاض بالقيم لكن لم تكن معنوية . بينما اظهر العد الكلي للكريات الدم البيضاء ، أظهر ارتفاعاً تدريجياً من خلال تأثير المستخلص ولكن لم يكن معنوياً. كما بينت نتائج كريات البيض التفريقي إلى زيادة الخلايا اللمفاوية ، قلة العدلات وقلة الحمضات والتي لم تكن معنوية. أظهرت المعلمات البيوكيميائية ، ان مستخلص *A. indica* المستويات طبيعية لإنزيمات المصل (AST و ALT و ALP) على الرغم من وجود انخفاض طفيف. ان البروتين الكلي كان ضمن المعدل الطبيعي. كما تشير القيم MCV و MCH و MCHC إلى حالة فقر الدم المعيارية. يعتبر مستخلص *A. indica* آمناً لخلايا الدم وإنزيمات الكبدو الكلى بجرعة أقل من 800 مجم / كجم من وزن الجسم.

Introduction

Many plants extracts are known to cause anaemia by destruction of RBCs, or may cause reduction in production of RBC in the bone marrow (1) and also cause liver and kidney damage. *A. indica* (Family, Meliaceae) known by common name Neem tree is native to Asian countries. It has long been used in India as remedy for sickness (2). The leaves, bark, stem, root have medicinal properties (3). *A. indica* is reported to be anthelmintic, antibacterial, antifungal, antihyperglycemic antiinflammatory, antiviral, antipyretic, insecticidal, hypercholesteremic and hypoglycemic agent (4,5). Chemical compounds in plants mediate their effect on the human/ animal body through processes identical with compounds in conventional drugs, thus herbal medicine do not differ greatly from conventional drug with reference to their mechanism of action (6). *Azadirachta indica* is called 'Ogwu akom' by Ibo tribe in Nigeria meaning literally malaria drug. The Hausa tribe in Nigeria call it *Dogo yaro*. Malaria is a major disease that kills children in Nigeria and other tropical countries. Environmental temperature provides conducive ground for the arthropod vector (mosquitoes) to thrive (7). Malaria is responsible for about 750000 mortalities especially in children. (8). AST and ALT elevation in conditions of hepatocyte damage in inflammatory condition of the liver, hypoxic states, hepatotoxicity by toxicants, trauma and some plant extracts, (9,10). Liver ALP elevation also in hepatocyte and biliary epithelial damage. They could also be ALP elevation in osteoblast, intestinal epithelial and corticosteroid stimulation when used for treatment (11, 12). Hyperproteinaemia is associated with dehydration occasioned by vomiting, diarrhoea, impaired renal concentration ability, excessive sweating or decreased water intake (13). Elevated urea production is associated with intestinal haemorrhage, increased dietary urea or increased protein catabolism, (14). Elevated creatinine occurs in pathological processes that cause a decrease in glomerular filtration rate which could be pre-renal, renal or post renal, (15). Hyperbilirubinaemia occur in diseases associated with haemolysis of blood as seen in babesiosis, anaplasmosis, trypanosomiasis, snake bite and some plant toxicants, (16). The aim of this work is to check

the effect of *A. indica* on haematological changes and biochemical indices for liver and kidney status after using the extract for treatment.

Materials and methods

Plant Materials

Leaves of *A. indica* were collected from the University environment in Umudike, Nigeria and was identified by Prof. M. C. Dike at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.



Picture of *Azadirachta indica* tree

Preparation of Plant Extract

The identified leaves of *Azadirachta indica* was dried under shade for 10 days and grinded to a coarse powder using manual grinder (Corona-Landers C 1A SA). Extraction was done by Soxhlet method described by (17,18) and 35g of coarse powdered sample was introduced into the extraction chamber using ethanol as solvent. Throughout the extraction time of 48 hours, the temperature was kept at 70⁰ C. The extract was concentrated in an oven at 30⁰ C and the dried extract weighed and kept in a labelled sterile specimen bottle for the work. Trial toxicity test showed that at 2000 mg/kg the rats were still alive and safe for use experiment. Thus different doses of 200, 400 and 800 mg/kg body weight was prepared and administered to the rats. These doses were calculated from a stock solution dissolved in distilled water.

Haematology and Biochemical Investigation

For Haematological screening, PCV was examined by the micro-hematocrit method as described by (19) using capillary tubes while RBC and WBC were counted manually using an improved Neubauer counting chamber. The differential leukocyte was counted manually using a thin blood film stained with Leizhman stain. (Hb) concentrations was determined by cyanomathemoglobin method, (20). Using RBC, PCV and (Hb), the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated using formulae;

$$\begin{aligned} \text{MCV} &= \frac{\text{PCV}}{\text{RBC}} \times 10 && \text{fl} \\ \text{MCH} &= \frac{\text{Hb}}{\text{RBC}} \times 10 && \text{pg} \\ \text{MCHC} &= \frac{\text{Hb}}{\text{PCV}} \times 100 && \text{g/dl} \end{aligned}$$

(21,22,23, 24).

Biochemical investigation was performed using ELISA reagent kits. The measure included, serum ALT, AST, ALP. (9,10).

Total protein was determined by Biuret method as described by (15). Samples were analysed immediately to avoid artifactual changes (25).

Experimental Animals

Male albino rats (140 to 250 g) were purchased from University Farm. Approval was obtained from College of Vet Medicine of the University in line with the guidelines for the care and use of laboratory animals as provided by National Research Council (26). The rats were acclimatized and fed *ad libitum*.

Experimental Design

Twenty rats were used for the research and were grouped into four of five rats each. Group 1 was the normal control group and was administered distilled water. Groups 2, 3, and 4 were the treatment groups which received 200, 400 and 800 mg/kg body weight of the *A. indica* extract respectively. The rats were dosed for 14 days, thereafter was sacrificed and blood collected by cardiac puncture for analysis. The effect of *A. indica* extract was checked on haematological parameters and serum enzymes activities.

Statistical Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20. Values were expressed as mean \pm Standard Error of Mean (SEM) and were further subjected to one - way analysis of variance (ANOVA) for comparison of doses with normal control. Duncan post-hoc test was used to separate the mean that showed significant difference. The statistical confidence was set at ($p < 0.05$).

Results and discussion

Fig 1 shows values presented as means \pm Standard Error of Mean (SEM) at ($p < 0.05$). RBC, PCV, Hb, TWBC.

There was progressive decrease in values of Hb, PCV and RBC when dose of the extract increased from 200, 400 and 800 mg/kg body weight.

Hb (g/dl) 16.0 ± 0.38 , 15.1 ± 0.38 , and 14.1 ± 0.38 , when compared to the normal control 17.0 ± 0.38 ,

PCV (%) 38.0 ± 0.82 , 37.5 ± 0.82 , and $34. \pm 0.82$, when compared to the normal control 39.0 ± 0.82

RBC ($\times 10^6 \text{ mm}^3$) 6.5 ± 0.13 , 6.0 ± 0.13 , and 5.5 ± 0.13 , when compared to the normal control 6.7 ± 0.13

The decrease in values of Hb, PCV and RBC was not statistically significant.

TWBC ($\times 10^3 \text{ mm}^3$) 9.0 ± 0.42 , 10.0 ± 0.42 , and 11.1 ± 0.42 , when compared to the normal control 8.5 ± 0.42 .

The mild elevation in values of TWBC recorded were not statistically significant at ($p < 0.05$).

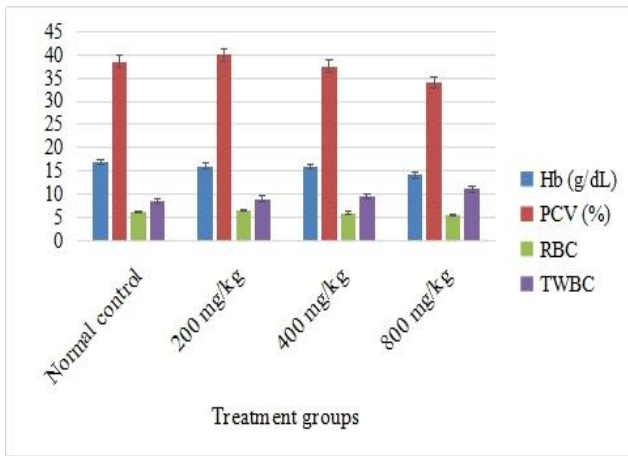


Figure 1: Haematology profile of Wistar rats.

Fig 2 shows values presented as means \pm SEM. MCV, MCH, MCHC were not affected significantly.

MCV(fl) 61.94 ± 0.18 , 62.63 ± 0.18 , and 61.68 ± 0.18 when compared to the normal control 61.60 ± 0.18

MCH (pg) 26.24 ± 0.20 , 26.26 ± 0.20 and 25.53 ± 0.20 , when compared to the normal control 25.62 ± 0.20

MCHC (g/dl) 42.36 ± 0.29 , 42.57 ± 0.29 , and 41.39 ± 0.29 , when compared to the normal control 41.59 ± 0.29

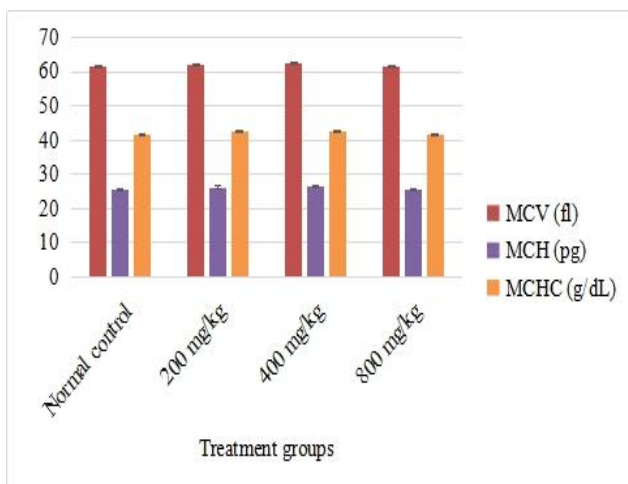


Figure 2: Haematology profile of Wistar rats.

The graph in Fig 3 represent the values of differential blood count of leukocytes as mean \pm SEM at significant difference ($p < 0.05$).

Lymphocytes (%) 56.75 ± 0.76 , 57.50 ± 0.78 , and 58.75 ± 0.76 , when compared to the normal control 56.25 ± 0.76

Neutrophils (%) 35.5 ± 0.79 , 33.25 ± 0.79 , and 34.75 ± 0.79 , when compared to the normal control 35.25 ± 0.79

Monocytes (%) 5.25 ± 0.39 , 5.00 ± 0.39 , and 5.00 ± 0.39 , when compared to the normal control 5.50 ± 0.39

Eosinophils (%) 2.50 ± 0.20 , 2.25 ± 0.20 , and 1.50 ± 0.20 , when compared to the normal control 3.00 ± 0.20

Basophils (%) 0.0 ± 0.00 , 00.0 ± 0.00 and 00.0 ± 0.00 , when compared to the normal control 0.0 ± 0.00

No statistical significant difference at ($p < 0.05$) and all values of leukocytes fall within normal reference range.

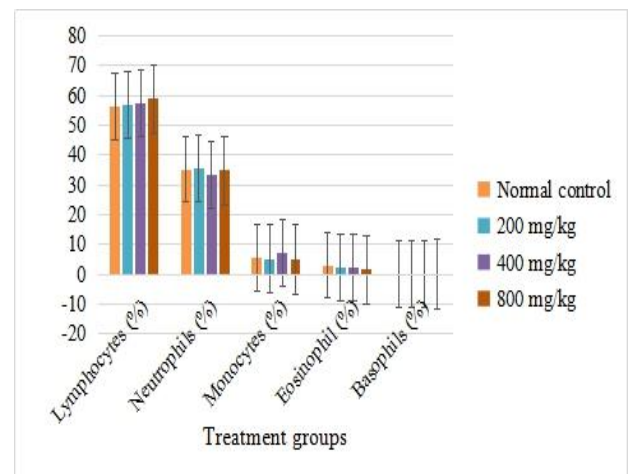


Figure 3: Differential leukocyte count

The graph in Fig 4 represent values of serum biochemistry of total protein, urea, creatinine and bilirubin. The value was represented as mean \pm SEM at ($p < 0.05$).

Total protein (mg/dl) 7.71 ± 1.12 , 7.21 ± 1.12 and 7.76 ± 1.12 , when compared to the normal control 7.8 ± 1.12 . (Ref range 4.0-8.0) (27)

Urea (mg/dl) 13.58 ± 0.55 , 14.98 ± 0.55 and

14.20 ± 0.55, when compared to the normal control 15.11 ± 0.55 (Ref range 10-30)

Creatinine (mg/dl) 0.79 ± 0.28, 0.89 ± 0.28 and 0.74 ± 0.28, when compared to the normal control 0.89 ± 0.28 (Ref range 0.6-1.6)

Bilirubin (mg/dl) 0.39 ± 0.01, 0.44 ± 0.01 and 0.38 ± 0.01, when compared to the normal control 0.44 ± 0.01 (Ref range 0-10)

No statistical significant differences at ($p < 0.05$) and all values were within normal reference range.

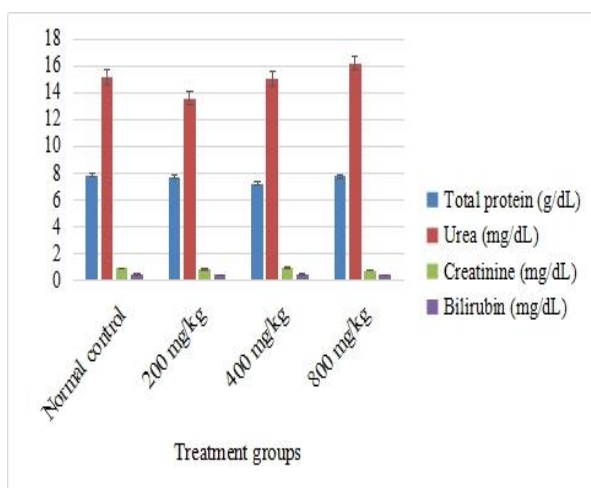


Figure 4: Serum biochemistry

The graph in Fig 5 represent the values of serum biochemistry of AST, ALT, and ALP at doses of 200, 400 and 800 mg/kg body weight. Values were represented as mean ± SEM at statistical confidence of ($p < 0.05$).

AST (μL) 40.99 ± 0.74, 40.25 ± 0.74 and 44.61 ± 0.74, when compared to the normal control 43.23 ± 0.74 (Ref range 32-84 μL)

ALT (μL) 29.02 ± 0.61, 27.16 ± 0.61 and 30.01 ± 0.61, when compared to the normal control 30.73 ± 0.61 (Ref range 30-58 μL)

ALP (μL) 93.19 ± 1.98, 96.11 ± 1.98 and 104.12 ± 1.98, when compared to the normal control 99.04 ± 1.98 (Ref range 0-500 μL)

No statistical significant differences at ($p < 0.05$) and all values fall within normal reference range.

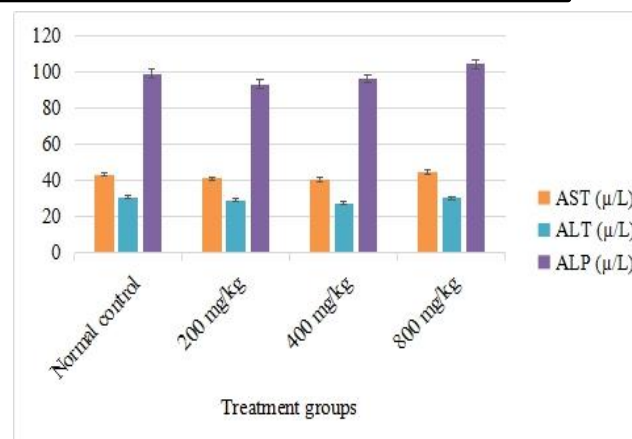


Figure 5: Serum biochemistry

Values are presented as means ± SEM. AST, ALT and ALP.

The haematological effect of *A. indica* extract on Wistar rats presented in Fig 1, showed mild decrease in Hb, PCV, and RBC mean value following administration of *A. indica* as the dose were increased from 200, 400 and 800mg/kg body weight, decreased but within normal range. From the result of this study decreases in Hb, RBC and PCV was not significant at ($p < 0.05$). There was mild decrease in TWBC value as the dose increased but was not significant at ($p < 0.05$). The red cell indices MCV, MCH and MCHC was normal meaning that *A. indica* extract even at the highest dose did not alter the size and haemoglobin concentration.

The percentage proportion of lymphocytes, neutrophils, monocytes and the basophils were not significantly ($p < 0.05$) varied in treated groups compared to normal control. Percentage proportion of eosinophil gradually decreased as the dose were increased and highest at 800 mg/kg body weight.

There were no significant differences at ($p < 0.05$) in values of different leukocytes counted though there was mild elevation Fig 3. Values of leukocytes fall within normal reference range.

Liver is vulnerable to attack because it is the major organ involved in biotransformation and detoxification the primary site of biotransformation and detoxification (28, 29). kidneys the principal organ for the excretion of waist products and osmoregulation is prone to

challenges by toxicants. When the cells of these organs are damaged it results in elevation of clinical Biochemical parameters in serum, such as AST, ALT and ALP or Creatinine, Urea as markers of impaired renal function, (30). The increase in these enzymes is indicated in hepatic and nephrotic disorders.

The result as presented in Fig 5, showed that the liver enzymes AST, ALT and ALP showed mild elevation but was not significant ($p < 0.05$). This observation suggests that *A. indica* extract was safe to liver or kidney which are responsible for metabolism, biotransformation, and elimination. Since *A. indica* did not cause any significant ($p < 0.05$) increase in serum total protein (TP), AST, ALT and ALP, of treated Wistar rats compared with the control rats, it could be seen in this work that *A. indica* extract at dose < 800 mg/kg body weight has no significant hepatotoxic or nephrotoxic effect on the liver and kidney respectively. This finding lay credence to the traditional claim of safety of the extract of *A. indica* to liver and kidney when used in the treatment of malaria at moderate doses < 800 mg/kg body weight.

The mild reduction in value of Total protein by this plant extract had no significant ($p < 0.05$), effect indicating its safety to the hepatosynthetic cells of the liver.

Conclusion

In this work, the extract of *A. indica* is safe to blood cells, liver and kidney at dose < 800 mg/kg body weight.

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