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

K.K. Igwe 1*, O. V. Ikpeazu 2 and I.E. Otuokere3

1Dept. of Vet. Biochem. and Animal Production, M. O. University of Agric. Umudike., Nigeria
2Dept of Biochem, Abia State University, Uturu, Abia State, Nigeria
3Depat of Chemistry, M. O. University of Agric. Umudike. Abia State, Nigeria

*Corresponding author; kkipwe191@gmail.com; drikpeazu@gmail.com

**Doi:** [https://doi.org/10.37940/AJVS.2020.13.1.15](https://doi.org/10.37940/AJVS.2020.13.1.15)

This article is licensed under a CC BY (Creative Commons Attribution 4.0)

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 neutropenia, 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.
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, antihyperglyceremic antiinflametory, 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). Hyperbilirubinemia 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.

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 70°C. The extract was concentrated in an oven at 30°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;

\[
\begin{align*}
\text{MCV} & = \frac{\text{PCV} \times 10^{6}}{\text{RBC}} \text{ fl} \\
\text{MCH} & = \frac{\text{Hb} \times 10^{6}}{\text{RBC}} \text{ pg} \\
\text{MCHC} & = \frac{\text{Hb} \times 100}{\text{PCV}} \text{ g/dl}
\end{align*}
\]

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 ± 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 ± 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. ± 0.82, when compared to the normal control 39.0 ± 0.82

RBC (X10\(^6\) 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 (X10\(^3\) 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 ± 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 ± 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, 0.00 ± 0.00 and 0.0 ± 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 ± 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.

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 800 mg/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.

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. 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 evaluation 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 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 lays 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.

References

1. Omoloye OA, Adedapo AA, Ohore OG. Studies on the toxicity of an aqueous extract of the leaves of Abrus precatorius in rats. Onderstepoort Journal of Veterinary Research. 2007 Mar 1;74(1):31-6.
2. Upadhyay UP, Vigyan PC. Neem (Azadirachta indica) and its Potential for Safeguarding. Journal of Biological Sciences. 2014;14(2):110-23.
3. Biswas K, Chattopadhyay I, Banerjee RK, Bandypadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica). CURRENT SCIENCE-BANGALORE-. 2002 Jun 10;82(11):1336-45.
4. Parotta JA. Healing plants of Peninsular India. CABI Publishing. pp 495-496. 2001.
5. Maragathavalli S, Brindha S, Kaviyarasi NS, Annadurai B, Gangwar SK. Antimicrobial activity in leaf extract of Neem (Azadirachta indica) Linn. Int J Sci Nature, 2012; 3: 110-113.
6. Vickers A, Zollman C, Lee R. Herbal medicine. West J 2001; 125:125-128.
7. Soulsby E.L. Helminths, Arthropods and Protozoa of Domestic Animals, Bailliere, Tindall and Cassell, 6th ed. London. 1976.
8. Greenwood D, Slack RC, Barer MR, Irving WL. Medical Microbiology E-Book: A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control. With STUDENT CONSULT Online Access. Elsevier Health Sciences; 2012 Jul 17.
9. Giannini E, Botta F, Testa E, Romagnoli P, Polegato S, Malfatti F, Fumagalli A, Chiarbonello B, Risso D, Testa R. The 1-year and 3-month prognostic utility of the AST/ALT ratio and model for end-stage liver disease score in patients with viral liver cirrhosis. The American journal of gastroenterology. 2002 Nov 1;97(11):2855-60.
10. Green RM, Flamm S. AGA technical review on the evaluation of liver chemistry tests. Gastroenterology. 2002 Oct 1;123(4):1367-84.
11. Cheung BM, Ong KL, Wong LY. Elevated serum alkaline phosphatase and peripheral arterial disease in the United States National Health and Nutrition Examination Survey 1999–2004.
12. Gowda S, Desai PB, Hull VV, Math AA, Vernekar SN, Kulkarni SS. A review on laboratory liver function tests. The Pan African Medical Journal. 2009;3.

13. Lubran MM. The measurement of total serum proteins by the Biuret method. Annals of Clinical & Laboratory Science. 1978 Mar 1;8(2):106-10.

14. Uchino S, Bellomo R, Goldsmith D. The meaning of the blood urea nitrogen/creatinine ratio in acute kidney injury. Clinical kidney journal. 2012 Apr 1;5(2):187-91.

15. Akanda MA, Choudhury KN, Ali MZ, Kabir MK, Begum LN, Sayami LA. Serum creatinine and blood urea nitrogen levels in patients with coronary artery disease. Cardiovascular Journal. 2013 Mar 27;5(2):141-5.

16. Krige JE, Beckingham IJ. ABC of diseases of liver, pancreas, and biliary system: liver abscesses and hydatid disease. BMJ: British Medical Journal. 2001 Mar 3;322(7285):537.

17. Cumpson P, Sano N. Stability of reference masses V: UV/ozone treatment of gold and platinum surfaces. Metrologia. 2012 Dec 19;50(1):27.

18. Jensen W. B. The origin of Soxhlex Extraction. Journal clinical Education, 2007; 84 (12):1913-1914.

19. Cole E.H. Veterinary Clinical Pathology. 4th ed. Saunders Company. Philadelphia PA, 1986.

20. Tietz NW. Carbohydrates In: Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, and Bruns DE. 2008.

21. Igwe K K, Udeh NE, Madubuike A J, Achi N K, Egwu L U, Ifenkwe D C, Mkpado CJ. Screening for Toxic and Haematological Changes of Ethanol Extract of Ficus capensis in male Wistar rats using some serum enzymes as Biochemical Markers. J. of Med. and Biol. Sci. Res. 2019; 5 (2) pp10-15.

22. Lewis SM, Bain BJ, Bates I, Dacie JV. Dacie and Lewis practical haematology. Churchill Livingstone; 2006 Apr 28.

23. Hoffbrand V, Moss P. Essential haematology includes free desktop edition. 6th Ed. John Wiley and Sons. 2011.

24. Igwe K K, Udeh NE, Madubuike A J, Egwu L U, Achi N K, Edet UE, Ifenkwe D C. Haematological and Toxicological Investigation of Ethanol Extract of Telferia occidentalis using some serum enzymes as Biochemical Markers in Wistar rats. J. of Med. and Biol. Sci. Res. 2019; 5 (1) pp 4-9.

25. NRC Guide for the care and use of laboratory animals. National Research Council, National Institute of Health. Bethesda (MD): pp 8523. 1985.

26. Ihedioha JJ, Onwubuche RC. Artifactual changes in PCV, hemoglobin concentration, and cell counts in bovine, caprine, and porcine blood stored at room and refrigerator temperatures. Veterinary clinical pathology. 2007 Mar;36(1):60-3.

27. Rodostits OM, Gay CC, Blood DC, Hincliff KW Veterinary Medicine 9th Ed. Saundier London pp 1819-1822.2000.

28. Diana Nicoll C. Appendix: Therapeutic drug monitoring and laboratory reference ranges. Current medical diagnosis and treatment. Stephen JM, Maxine AP. 46th edition, Mc Graw hill. 2007:1767-75.

29. Thapa BR, Walia A. Liver function tests and their interpretation. The Indian Journal of Pediatrics. 2007 Jul 1;74(7):663-71.

30. Robert O, Michael S, Disha M, Dvorah H, Abu S, Michael S, Thomas K, Sanjay D, Daniel, S and Yunling D. The Association of Blood Urea Nitrogen Levels and Coronary Artery Disease. The Einstein Journal of Biology and Medicine 2010; 3-7.