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THE EFFECT OF AQUEOUS EXTRACT OF NEEM (*AZADIRACHTA INDICA)* LEAVES ON LIVER FUNCTIONS OF WISTAR RATS

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ABSTRACT
Medicinal plants are part of human society to combat diseases. Azadirachta indica evidently has great medicinal potentials. This work was undertaken to investigate the morphological and some enzymatic effect of A. indica extract on the tissues of the liver. Twenty four (24) adult Wistar rats of both sexes, average weight, 195g were randomly divided into four (4) groups of Control group (n = 6), and three (3) test groups of 6 rats each. The test groups (A,B,&C) were administered 750mg/kg, 500mg/kg and 250mg/kg respectively, through an orogastric tube, for 28 days consecutively. After which the animals were sacrificed and the liver was excised and processed for histological examinations. Blood samples were collected for Biochemical analysis. A. indica stimulates production and storage of proteins in the liver. These changes are dose-dependent. This study suggests that A.indica extract increases cellularity, immunostimulant activity, boosts the Mononuclear Phagocyte System, and confers hepato protection as well.

INTRODUCTION

Azadirachta Indica, (A. Indica) normally found in the Indian subcontinent and the dry forest areas of south and Southeast Asia including Pakistan, Srilanka, Thailand, Malaysia and Indiansia, but cultivated in most other countries of the world belongs to the plant family meliaceae [5,11]. Review of the biological activities and medicinal properties of the plant revealed the antimalarial and hepatoprotective activities of its leaves, stem, bark and seed oil [2]. The mononuclear phagocyte system (sometimes called the reticuloendothetial system) consists of closely related cells of bone marrow origin, including blood monocytes and tissue macrophages [9]. The latter are diffusedly scattered in the connective tissue or located in organs such as the liver (Kupffer cells), spleen, lymph nodes (Sinus histocytes) and lungs (alveolar macrophages) [10]. Mononuclear phagocytes arise from a common precursor in the bone marrow, which gives rise to blood monocytes, this migrate into various tissues and differentiate into macrophages [8]. The products of activated macrophages serve to eliminate injurious agents such as microbes and to initiate the process of repair, responsible for much of the tissue injury in chronic inflammation [10].While questions still remain about the dosage required in human beings. Neem clearly has great potential in preventing malaria, an infection that affects 300 million and kills 1 (one) million people each year [10]. Neem seed and leaf extracts are effective against Malaria parasites. Components of the alcoholic extracts of leaves and seeds are effective against both chloroquin-resistant and sensitive strains of Malaria parasite. Recently, neem seed extract and its purified fractions have been shown to inhibit growth and development of sexual and asexual stages of drug

KEYWORDS: A*zadirachta Indica, Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP)*

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sensitive and resistant strains of the human Malaria parasite plasmodium falciparum. This work aims at investigating the morphological and biochemical effects of the aqueous extracts of A. indica administered to normal rats in different doses on the tissues of organs of the Mononuclear phagocyte system.

MATERIALS AND METHODS

The Neem (Azadirachta Indica) leaves used in this study were collected from around the University of Benin Main Campus in 2011 and identified by a Botanist in the Department of Plant and Biotechnology, University of Benin, Nigeria.

The fresh matured leaves collected were air-dried in the laboratory (to prevent solar leaching) and grounded into powder using wooden pestle and mortar to obtain 4.0kg powder, which was exhaustively extracted in distilled water for 8 hours at 60°C using soxhlet extractor (Quickfit, England), by technicians in the Department of Pharmacology, University of Benin, Nigeria. The Neem leaf extract was concentrated on an aluminum tray, placed in an oven and maintained overnight at 60°C to dry, then stored at room temperature (27°C) until used.

Twenty-four (24) Adult Wistar rats of both sexes, weighing 150-240 g were randomly separated into four (4) groups; Control, A, B, & C, of six rats each. Group A: Rats were given 750mg/kg extract, group B rats were given 500mg/kg of the aqueous extract. and group C rats were given 250mg/kg of the aqueous Neem leaf extract. Each group had access to normal animal feed and water.

All animals were treated in accordance with the Guide for the care and use of laboratory animals prepared by the National Academy of Sciences and Published by the National Institute of Health Guide for the use of Laboratory Animal (NIN, 2002 Production, No. 83-23), Revised 1978.
The assay methods are for the quantitative in vitro determination of the enzymes (ALT, AST, ALP) in serum. AST and ALT estimation was done by monitoring the concentration of pyruvate hydrozone formed from 2, 4-dinitrophenylhydrazine [1], ALP estimation was done using phenolphthalein Monophosphate method [7], and histological procedure followed for the harvested tissues using Carleton's method [3].

STATISTICAL ANALYSIS

Biochemical quantitative in vitro determination of the enzymes, were analyzed for statistical significance by one-way ANOVA using the statistical packages for Social Sciences (SPSS) and Post Hoc Test between groups using Microsoft Excel Programme.

The analyzed data were represented in tables and charts, where applicable. Descriptive statistics were obtained for all variables documented. All data were expressed as Mean ± SEM. The confidence limit set at 95% and probability value of $P < 0.05$ was regarded as significant.

RESULT

The value of Aspartate Aminotransferase (AST) level in the control and experimental groups (groups A, B and C) is 16.00 ± 0.0IU/L, 36.67 ± 11.00IU/L, 45.33 ± 12.4IU/L and 45.00 ± 12.4IU/L respectively.

The value of Alanine Aminotransferase (ALT) level in the control and experimental groups (groups A, B and C) is 2.00 ± 0.0IU/L, 8.50 ± 1.8IU/L, 14.33 ± 4.3IU/L and 24.00 ± 2.7IU/L respectively.

The value of Alkaline Phosphatase (ALP) level in the control and experimental groups (groups A, B and C) is 58.00 ± 0.0IU/L, 98.67 ± 14.9IU/L, 82.33 ± 12.4IU/L and 97.83 ± 14.3IU/L respectively.

There was an increase in the level of AST and ALT level as the dose decreases but the level of ALP shows an initial increase in group A, administered the low dose while decrease along the groups but was not statistically significant.

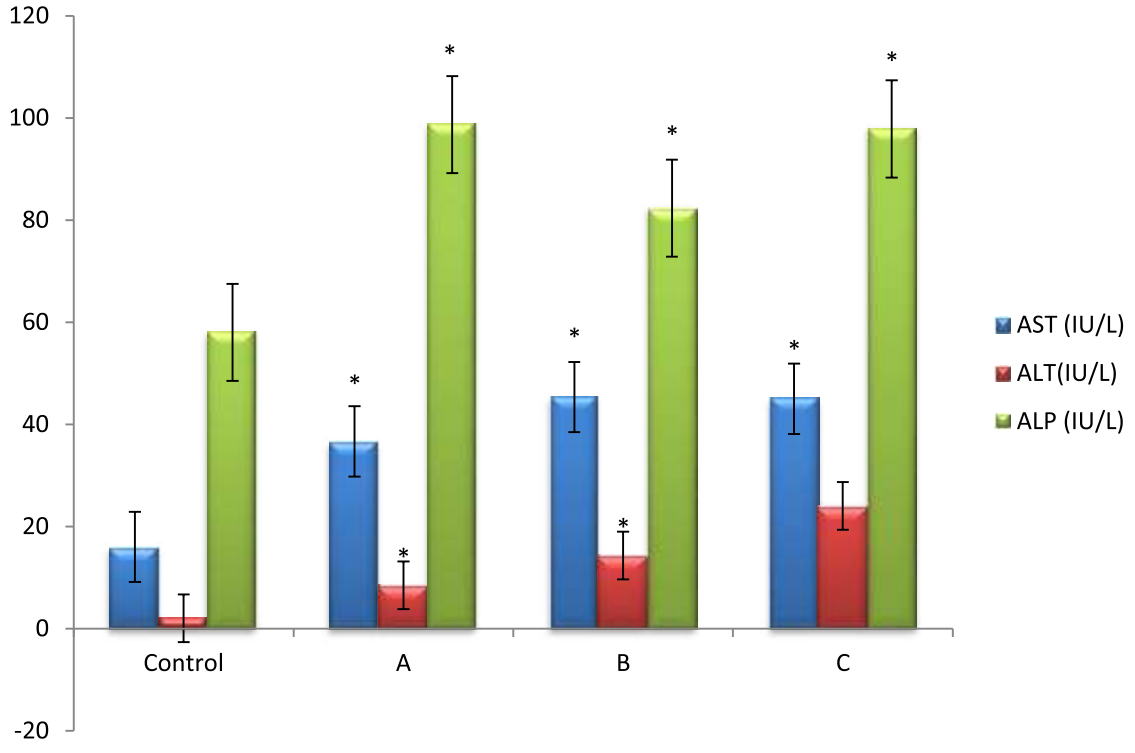
It was however observed that the increase in the experimental groups when compared to the control group showed a statistically significant difference as shown in the table and figure below.

TABLE : Summary of Biochemical changes induced by a. indica on the ALT, AST AND ALP levels among the groups of Adult Wistar rats.

GROUPS	AST (IU/L)	ALT(IU/L)	ALP (IU/L)
Control	16.00 ± 0.0	2.00 ± 0.0	58.00±0.0
A	36.67 ± 11.00*	8.50± 1.8*	98.67±14.9*
B	45.33 ± 12.4*	14.33 ± 4.6*	82.33±12.4*
C	**45.00 ± 12.4***	**24.00 ± 2.7***	**97.83±14.3***

* significant at P ≤ 0.05; A = High Dose; B = Moderate Dose; C= Low Dose

Fig. 1: Blood Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) level after 28 days treatment with extract: *A. indica* in adult Wistar rats.



* significant at P ≤ 0.05; A = High Dose; B = Moderate Dose; C= Low Dose

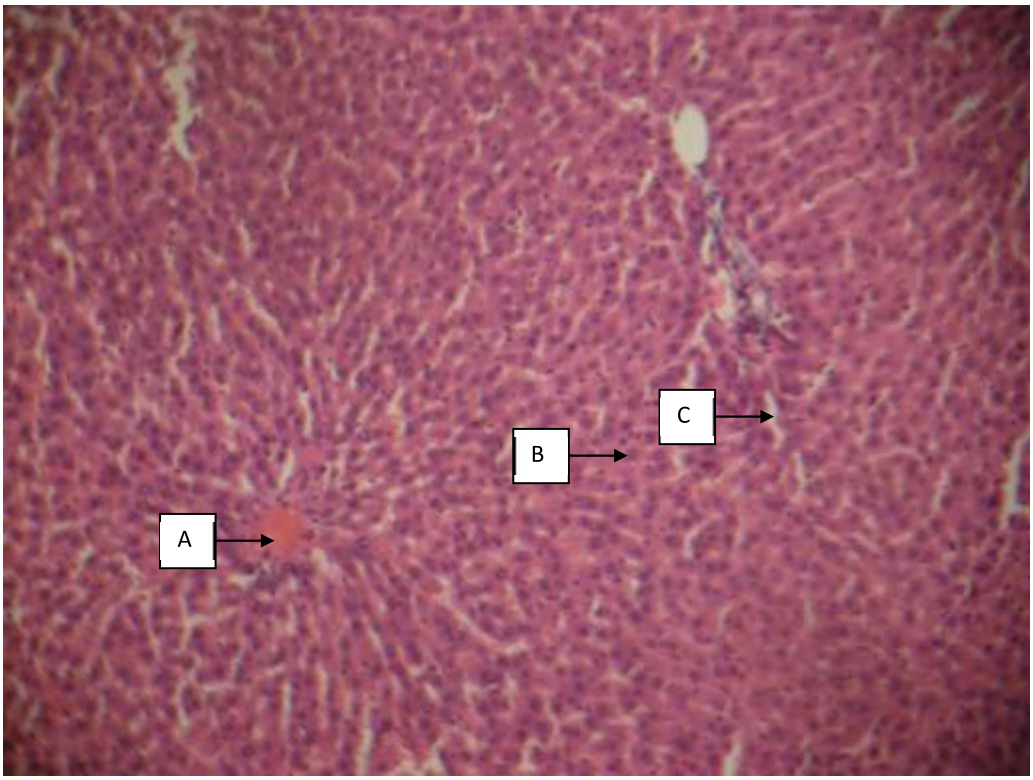


Fig 2: Normal Rat Liver Showing Portal Triad A, Hepatocytes B and Sinusoids C. (H&E x 4)

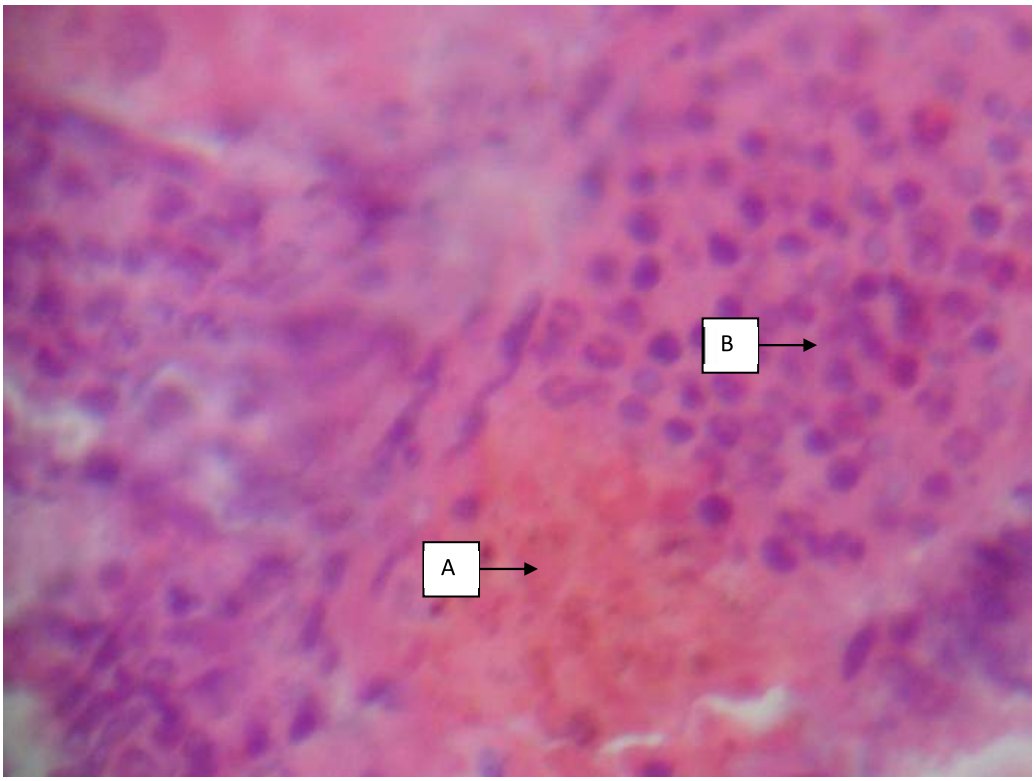


Fig 3: Rat Liver Treated with High Dose Azadirachta indica showing Portal Congestion A, and Moderate Periportal Infiltrates of Lymphocytes B (H&E x 10)

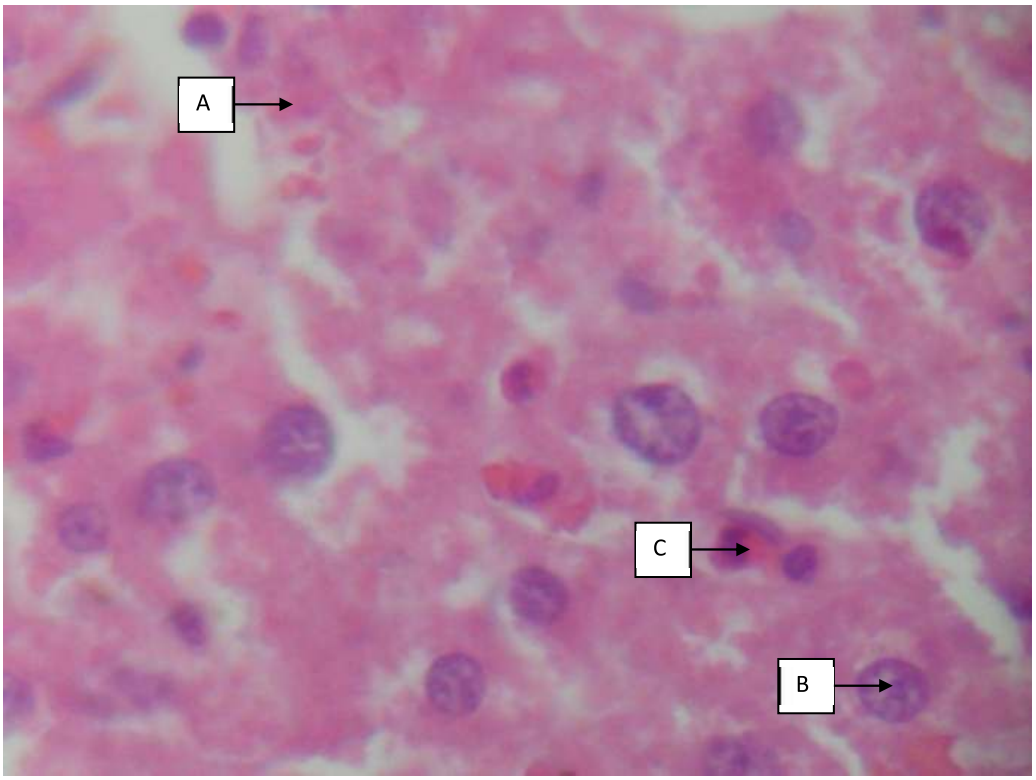


Fig 4: Rat Liver Treated with High Dose Azadirachta indica showing Sinusoidal Congestion A, Prominent Nucleoli B and Kupffer Cell Hyperplasia C (H&E x 40)

DISCUSSION

Our present investigation has demonstrated the effects of aqueous extract of A. indica (Neem) on the liver.

Modern clinical studies have identified a number of compounds in the Neem tree that effectively regulate Immune system functions[4].

The abnormal levels of the liver transaminases and evidence of liver damage, usually manifest as a result of architectural disarray, vascular congestion, hepatocytes necrosis, apoptosis, or inflammatory cell infiltration in either acute or chronic conditions. None of these features were observed in the rats administered with the highest dose of the extract, except for moderate infiltration of the portal area by lymphocytes as shown in figure 3. This study demonstrates the hepatoprotective activity of A. indica [2]. Generally, cells die as a result of necrosis or apoptosis when they are challenged with toxins, noxious agents or injuries[6].

Although mild effect of this extract to some extent showed as in the figure above on liver enzymes-Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline Phosphatase (ALP) which was negligible.

Correlating the Microscopic architecture of the organs in the test groups with the liver enzymes Biochemical analysis, our investigation suggests relatively, that A. indica does not inflict serious organ damage in relatively normal rats.

CONCLUSION

This work has demonstrated that aqueous extract of Neem Azadirachta indica to an appreciable extent, does not have any hepatotoxic effect nor any cholestotic effect at the 750mg/kg, 500mg/kg and 250mg/kg doses given for 28 days consecutively.

More research is needed to corroborate this investigation most especially the morphological and biochemical implications. As well as Azadirachta indica clinical and therapeutic utilization.

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