

EFFECT OF ETHANOLIC EXTRACT OF LEAF OF AZADIRACHTA INDICA ON SOME HEMATOLOGICAL PARAMETERS IN ALBINO WISTER RATS

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ABSTRACT

The aim of this study was to evaluate the effects of ethanolic extract of the leaf of *Azadirachta indica* on haematological indices of rats. Twenty four adult male rats (weighing between 100 and 120g) were randomly but equally divided into four groups of six rats per group. Rats in group 1 (control) were administered with 10ml/kg of distilled water while rats in groups 2 to 4 were respectively administered with the extract of *A. indica* orally at the dose levels of 100mg/kg, 200mg/kg and 300mg/kg, once daily for 14 days. On the 15th day post administration, rats of all the groups were sacrificed and their blood samples were collected through cardiac puncture into EDTA sample bottles for haematological analysis. The results showed that PCV, Hb, RBC counts were non-significantly increased in all the experimental groups compared to control, indicating that extract of *A. Indica* caused non-significant increase in the PCV, Hb and RBC counts. The mean white blood cell and platelet counts were significantly decreased in group 3 compared to control. The mean lymphocyte values increased while the mean neutrophil values decreased in all the experimental groups compared to control. The mean values of MCH, MCHC and MCV were similar between experimental groups and the control. Therefore, in conclusion low dose of ethanolic *Azadirachta indica* causes increase in the cellular components of blood but higher doses may result in decrease of some or all of these blood parameters.

KEYWORDS: *Azadirachta indica*, extract, white blood cell, platelet, lymphocyte.

INTRODUCTION

Medical plants have been part of human society to combat diseases, from the dawn of civilization. History shows that the Sumerian (5,300B.C) used herbs as medicine. Many of the drugs used today were obtained from plants. These include castor, Rauwolfia, Aloe, foxglove. Despite these facts, interest in medical plants began to decline and attention was shifted to synthetic drugs which appeared more potent (despite it's toxicity). This trend had continued

especially in the technologically advanced countries until recently. Currently WHO estimates that 80% of the developing countries medical plants 25% in the developed

A. indica is well known in India and neighbouring countries for more than 200years as one of the most versatile medicinal plants having a wide spectrum of biological activity. Every part of the plant has been used as traditional medicine for household remedy against, various human ailments from antiquity (Chopra et al, 1958; Chatterjee et al 1994). The

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tree is regarded as "village dispensary" in India. The importance of the neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled "Neem, a tree for solving global problems". *Azadirachta indica*, commonly called neem or dogonyaro is a plant that has found varied use in ecological, medicinal and agricultural sectors. Biological and pharmacological activities attributed to different chemical compositions of the plant extracts are numerous. Alcoholic extracts of the leaves and seeds of *A. indica* have been shown to be effective against chloroquine resistant and sensitive strains of malaria parasites (Badani et al, 1987).

Compounds isolated from various parts of the plants are Nimbidin, ninbin, numbinin, nimbidinin nimbolide and nimbidic acid (Mitra et al 1971). In recent times, the consumption of *Azadirachta indica* has increased, also the possibility of its abuse and the attendant toxic effect on physiological systems (Sara 2004). Many researchers have shown that this plant is immunostimulatory (Uphadhyay et al 1992). However reports on red blood cell count and indices have been inconclusive. Thus this study was conducted to assess the effects of this plant on red blood cell index and other haematological parameters.

Materials/Methods

The fresh leaves of *A. indica* were collected from a forest in Aka, Uyo Local Government Area, Akwa Ibom State, Nigeria. They were authenticated by Dr. (Mrs.) Margaret Basse of the department of Botany, university of Uyo, Uyo, Akwa Ibom State, Nigeria. The leaves were shredded, dried and pulverized. 800g of the powdered sample was macerated in ethanol for 72 hrs after which it was filtered. The filtrate was then evaporated in water bath to dryness to obtain a solid extract. Twenty four adult male albino rats, weighing between 100 and 120g were obtained from animal house unit of department of pharmacy, university of Uyo, Nigeria. The animals were kept in environmentally adapted wooden cages with woods shaving as their beddings and were allowed for 1 week to acclimatize before the commencement of research. The room temperature was 25 – 29°C; 12 hours day light and 12 hours dark cycle was maintained. The animals were kept in dry and hygienic conditions and fed on standard rats pelleted diet with water given *ad libitum*.

Twenty four male rats were divided into four groups of six rats per group. Groups 2, 3, and 4 received orally 100mg/kg/d, 200mg/kg/d, and 300mg/kg/d of the neem extract respectively for 14 days while group1 received 10ml/kg of distilled water as control. After 21 days of treatment the rats were anaesthetized using chloroform. Blood samples were collected through cardiac puncture, using 5ml syringe and put in EDTA sample bottles. Packed cell volume, hemoglobin concentration, RBC counts, MCV, MCHC, MCH, WBC (total and differential) and platelet counts were determined using KX-21 haematology analyzer.

RESULTS

The mean total white cell counts of rats in the control group ($13.58 \pm 1.89 \times 10^3 / \mu\text{l}$) were significantly higher compared to rats in group4 ($11.25 \pm 1.03 \times 10^3 / \mu\text{l}$) but non-significantly lower than group3 ($17.43 \pm 0.99 \times 10^3 / \mu\text{l}$). Lymphocyte counts were significantly higher in all the experimental groups: group2 (83.58 + 1.03%), group 3 (76 ± 1.47%) and group4 (81.85 + 2.02%) compared to control (P<0.05). Neutrophil counts were significantly lower in all the experimental groups: group2 (16.43 + 1.03%) group3 (24 ± 0.77%) and group4 (18.15± 2.2%) compared to control.

Platelets counts were significantly higher in group2 ($856.25 \pm 1.0^3 / \mu\text{l}$) and group3 ($862.5 \pm 0.96 \times 10^3 / \mu\text{l}$) but lower in group4 ($720.25 \pm 0.63 \times 10^3 / \mu\text{l}$) compared to control group ($730.25 \pm 0.63 \times 10^3 / \mu\text{l}$).

The mean Pcv values were non-significantly higher in all the experimental groups: group2 (44.3+0.6%), group3 (47.25 + 1.89%) and group4 (43.96± 1.26%) compared to control (42.83±2.69%).

The mean Hb concentration was also non-significantly higher in the experimental groups: group2 (12.08 ± 0.27g/dl), group3 (13.03 ± 0.69g/dl), and group4 (12.13 ± 0.4g/dl) compared to the control group (11.75± 0.79g/dl). The total red blood cell counts were non-significantly higher in group3 ($7.74 \pm 0.45 \times 10^6 / \mu\text{l}$) compared to control group ($6.83 \pm 0.26 \times 10^6 / \mu\text{l}$). The mean MCV values were similar between the control (61.68±1.95fl) and the experimental groups (64.63±0.35fl, 61.28±1.42fl, 64.68±1.67fl respectively). The mean MCH values were similar between the control (16.93±0.57pg) and the experimental groups (17.63±0.35pg, 16.88±0.45pg, 17.88±0.82pg respectively). The mean MCHC values between the experimental groups (27.23±0.25g/dl, 27.5±9.35g/dl,

27.5±0.69g/dl respectively) and that of the control (27.4±0.21g/dl) were similar.

Table 1: Haematological responses (mean ± SEM) in Wistar rats following administration of ethanolic extract of Azadirachia for 14 days.

Groups	PCV (%)	Haemoglobin (g/dL)	RBC (x10 ⁶ /μL)	Red blood cell indices		
				MCV (fL)	MCH (pg)	MCHC (g/dL)
G1 (Control)	42.83±2.69	11.75±0.79	6.83±0.26	61.68±1.95	16.93±0.57	27.4±0.21
G2 (100mg/kg)	44.3±0.62	12.08±0.27	6.86±0.12	64.63±0.8	17.63±0.35	27.23±0.25
G3 (200mg/kg)	47.25±1.89	13.03±0.63	7.74±0.45	61.28±1.42	16.88±0.45	27.5±9.35
G4 (300mg/kg)	43.96±1.26	12.13±0.43	6.81±0.27	64.68±1.67	17.88±0.82	27.5±0.69

Table 2: haematological responses (mean ± SEM) in Wistar rats following administration of ethanolic extract of Azadirachia indica for 14 days.

Groups	WBC (x10 ³ /μL)	Platelets (x10 ³ /μL)	Lymphocytes (%)	Neutrophils (%)
G1 (control)	13.58±1.89	730.25±0.63	63.35±1.23	34.6±1.1
G2 (100mg/kg)	13.43±3.08	856.25±1.03 ^a	83.58±1.03 ^a	16.43±1.03 ^a
G3 (200mg/kg)	17.43±0.99	862.5±0.96 ^{a,b}	76±1.47 ^{*,f}	24±0.77 ^{a,d}
G4 (300mg/kg)	11.25±1.03 ^e	720.25±0.63 ^{a,b,c}	81.85±2.02 ^a	18.15±2.2 ^{a,e}

a = significantly different from G1 (p<0.001)

b = significantly different from G2 (p<0.001)

c = significantly different from G3 (p< 0.001)

d = significantly different from G2 (p<0.01)

e = significantly different from G3 (p<0.05)

f = significantly different from G3 (p<0.05)

** = significantly different from G1 (p< 0.01)

DISCUSSION

This ethanolic extract of *Azadirachta Indica* stimulated increase in circulating lymphocytes. This increase in circulating lymphocyte may result in enhanced immunological status of the body (Guyton and Hall, 2006), especially the cell mediated immune response (Lowenthal et al; 1994). This immunostimulatory effect of *A. Indica* is probably the reason for its wide range medicinal properties. The percentage of circulating neutrophils was significantly reduced. Neutrophil counts are usually increased in acute bacterial infections. This finding, therefore suggests that *A. indica* may not be suitable for acute bacterial infections.

The platelet counts were also significantly higher in the experimental group compared to the control group. This stimulatory effect of *A. Indica* on platelet counts has also been reported by many researchers (Ghosh et al 2006). This plant has also been shown to reverse or prevent cyclophosphamide induced

thrombocytopenia both in normal and tumor bearing mice (Ghosh et al 2006).

There was a non-significant increase in red cell count, Pcv and Hb. Our finding is in support of an earlier work by Parshad et al, 1994. The increase in Pcv may be as a result of increased cellular components of the blood. Though the exact mechanism is not known, it is possible that *A. Indica* acts by enhancing the division of pluripotent uncommitted stem cell into various cell lines. Some researchers have suggested that many of the effects of *A. Indica* are attributed to synergistic actions of different constituents of the plant (Badani et al, 1987). The stimulatory effect on haematological parameters was most marked at the dose of 200mg/kg. However, at the dose of 300mg/kg, there was significant reduction in total white cell and platelet count. It appears from these observations that the optimal stimulatory dose of this extract with regards to red cell count, Pcv, Hb, WBC and RBC count is 200mg/kg, and that higher doses may actually cause reduction of some or all the parameters. This could have

actually been the case with an earlier study (Ali et al 1987) which reported a decrease in all the cellular components of blood, following the administration of *A. Indica* extract

In conclusion, ethanolic extract of *Azadirachta indica* in low dose stimulates haematopoiesis while higher doses are haematotoxic.

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