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CHANGES IN THE HAEMATOLOGY, SOME SERUM BIOCHEMICAL PARAMETERS AND HISTOPATHOLOGY OF WISTAR RATS EXPOSED ORALLY TO METHANOLIC EXTRACT OF THE ROOT BARK OF *AFRORMOSIA LAXIFLORA*.

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ABSTRACT

Afromosia laxiflora Benth is a medicinal plant that has been used for the treatment of various ailments of human and animals over the time. It has been found to be useful in the treatment of pain, diarrhoea, dysentery, gout and also as antidepressant and anticonvulsant. In this study, methanolic extract of the root bark of this plant was administered orally to 3 groups of rats containing 6 rats in each group at 250, 500 and 1000 mg/kg body weight respectively while the control group was administered with distilled water orally. The administrations were done for 21 days and blood was thereafter obtained from the anaesthetized rats through cardiac puncture. The results of the blood and serum analyses showed a decrease in red blood cell count, haemoglobin concentration and an increase in total white blood cell and lymphocytes. The total protein slightly decreased but alanine aminotransferase and aspartate aminotransferase significantly ($p<0.05$) increased. Some pathological changes were also observed in the liver, kidney and the lungs. It was therefore suggested that the extract could be toxic to the liver as the aminotransferases significantly increased and increase in white blood cell could possibly help the immunity of the rats.

KEYWORDS: *Afromosia laxiflora*, root, extract, rats, blood, serum.

INTRODUCTION

The importance of medicinal plants in traditional health care systems of the world is gaining increased attention; large number of people in many countries of the world depends on medicinal plants for their health care delivery due to low cost, relative safety (Kala 2005) and abundance of these plants naturally in their environment. Although, medicinal plants are often been promoted by the traditionalists to the public as been natural and completely safe relative to conventional medicines (Adewunmi and Ojewole 2004), however, it has been discovered that some of these plants used medicinally and widely, and assumed to be safe are potentially toxic due to the presence of toxic and potentially lethal constituents in them (Fennel *et al*, 2004) thereby posing danger to the users. *Afrormosia laxiflora* Benth of the family Leguminosae is a medicinal plant that was highly acclaimed for its healing effects in the treatment of various diseases of human and animals. It has been found to be useful in the treatment of pain, diarrhoea, dysentery, gout, as antidepressant and anticonvulsant (Haruna 2000) either alone or in combination with other herbal materials, the activities of this plant for the treatment of insomnia, epilepsy and anxiety (Bum *et al*, 2011) have also been reported. Its antifertility (Rashmi *et al*, 2014) activity has also been used traditionally. On phytochemical analysis, α-methyldeoxybenzoins angolensin, 2-O-methyl-angolensin and the pterocarpan maackiain (demethylpterocarpin) have been isolated by bioactivity-guided fractionation (Corrado 1995). Although, medicinal plants are widely used and assumed to be safe and their toxicities often neglected, however, they can be toxic thereby posing health risk to the users, the need for the study of their toxicity is therefore of paramount importance and hence this study.

MATERIALS AND METHODS

Animals and experimental design: The animals used in this study were male Wistar albino rats weighing between 100 and 170 grams, obtained from National Veterinary Research Institute Vom, Plateau State Nigeria. The animals were maintained at the Animal House of the Faculty of Veterinary Medicine, University of Abuja. They were kept in rat cages and fed with rat pellets (Growers' mash, Vital feed® Nigeria) and allowed free access to clean fresh water in bottles *ad libitum*.

Twenty four animals divided into 4 groups A, B, C and D of 6 animals per group were used in this study. While the first 3 groups A, B and C were administered orally with 250, 500 and 1000 mg/kg body weight of the plant extract, the fourth group D served as control and was

administered orally with 3 ml/kg of distilled water. The plant extract and distilled water was administered to the respective group by oral gavage for 21 days and blood samples were thereafter obtained from the anaesthetized rats through cardiac puncture for analyses. The rats were humanely sacrificed and sections of the liver, kidneys and lungs were processed for histopathological examination.

Preparation of the methanolic extract of the root bark of the plant: Plenty bark of the root of *Afrormosia laxiflora* were obtained and washed properly with clean water, cuts into small pieces and air dry for two weeks to get very dried sample, it was then pounded with pestle and mortar to fine particles. 500g of the powdered sample was added to 1 liter of methanol (80% v/v) for 72 hrs with intermittent shaking at 2 hrs interval. Thereafter, the solution obtained was filtered using filter paper and the filtrate which is the extract was concentrated using vacuum rotary evaporator at 4^0C.

Technique for obtaining blood and serum samples: Blood samples were collected under light ether anaesthesia by cardiac puncture into heparinized bottles for haematological studies. A further blood sample was collected into a clean bottle (non-heparinized) and allowed to clot. The clotted blood with serum was centrifuged at 3000 rpm for 5 min. Serum samples were collected into clean bottles for biochemical analysis.

Determination of haematological parameters: Blood samples collected from rats into heparinized bottles were analyzed for the determination of packed cell volume (PCV), red blood cell (RBC) count, haemoglobin concentration, platelet count, total and differential white blood cell (WBC) count using standard methods (Ghai, 1995).

Determination of serum biochemical parameters: the serum collected was analysed for total protein using biuret reaction while albumin was measured by colorimetric estimation using the sigma diagnostics albumin reagent (Sigma Diagnostic, U.K.) which contained bromocresol green (BCG). Globulin was measured from the difference between total protein and albumin. Alanine aminotransferase (ALT) was measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenyl hydrazine while aspartate aminotransferase (AST) was determined by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenyl hydrazine.

STATISTICAL ANALYSIS

The entire data collected was statistically analysed using one-way ANOVA and Duncan New Multiple Range post hoc test from SPSS 16.0 package to compare the mean values of the test groups with the control.

RESULTS

The extract obtained was dark brown in colour with pasty consistency and the total solid recovered from the extract was 9.87 percent (w/w).

The result of the blood analysis showed a non-significant decrease in red blood cell count and haemoglobin concentration of the group administered with 1000 mg/kg of the extract compared with the control group, while the packed cell volume and mean corpuscular volume of groups 500 mg/kg and 1000 mg/kg respectively increased significantly ($p<0.05$) as shown in table 1.

Table 1: Haematological parameters (Mean±SEM) of rats exposed to methanolic root bark extract of *Afrormosia laxiflora*.

Treatment	RBCx10^6/µl	PCV(%)	HB(g/dl)	MCV(fl)	MCH(pg/cell)	MCHC(%)
250mg/kg	4.42±0.62	33.33±3.52	12.90±1.30	51.70±10.41	29.84±1.53	37.98±7.24
500mg/kg	4.86±0.42	38.67±1.74*	15.58±0.40	72.66±10.23	31.28±3.29	42.59±1.22
1000mg/kg	3.90±0.21	32.67±1.57	11.07±1.54	83.13±4.06*	26.26±2.51	32.40±4.63
Dist H_2O (3ml/kg)	5.58±0.23	33.67±1.16	15.43±0.66	60.22±2.56	27.30±1.23	45.28±0.64

*significant difference at $p<0.05$ between the test and control groups.

The table 2 showed a significant increase in total white blood cell and lymphocytes of the group administered with 100 mg/kg of the extract compared with the control group.

Table 2: Total white blood cell and differentials (Mean±SEM) of rats exposed to methanolic root bark extract of *Afrormosia laxiflora*.

TreatmentMono(%)	TWBCx10^3/µl	Neut(%	Lymp(%)	Eosin(%)	Baso(%)
250mg/kg	2.01±0.17	41.00±0.2	0.00±0.00	1.33±0.25	2.00±0.41
500mg/kg	2.62±0.13	41.33±0.25	0.67±0.20	0.33±0.25	0.67±0.25
1000mg/kg 1.67±0.25	2.94±0.31*	39.33±1.70	57.67±0.40*	0.67±0.20	0.67±0.20
Dist H_2O (3ml/kg)	1.84±0.12	46.67±0.40	0.67±0.20	1.67±0.25	2.67±0.37

*significant difference at $p<0.05$ between the test and control groups.

The serum biochemical analysis showed the total protein that slightly decreased, but alanine aminotransferase and aspartate aminotransferase significantly ($p<0.05$) increased for the groups administered with 500 and 1000 mg/kg of the extract compared with the control as shown in table 3.

Table 3: Some serum chemistry parameters (Mean±SEM) of rats exposed to methanolic root bark extract of Afrormosia laxiflora.

Treatment	ALT(iu/l)	AST(iu/l)	T. Prot(g/dl)	Albu(g/dl)	Glob(g/dl)
250mg/kg	26.72±0.48	79.29±2.36	7.74±0.16	4.00±0.10	3.74±0.15
500mg/kg	32.70±1.23*	105.24±4.33*	7.00±0.00	4.00±0.09	3.01±0.01
1000mg/kg	36.35±1.47*	122.52±3.69*	6.56±0.11	3.73±0.18	2.89±0.46
Dist H_2O (3ml/kg)	23.74±0.85	62.21±1.86	8.60±0.18	4.00±0.19	4.51±0.28

*significant difference at $p<0.05$ between the test and control groups.

The histopathology also showed some slight pathological changes in the liver, kidneys and the lungs.

HISTOPATHOLOGY

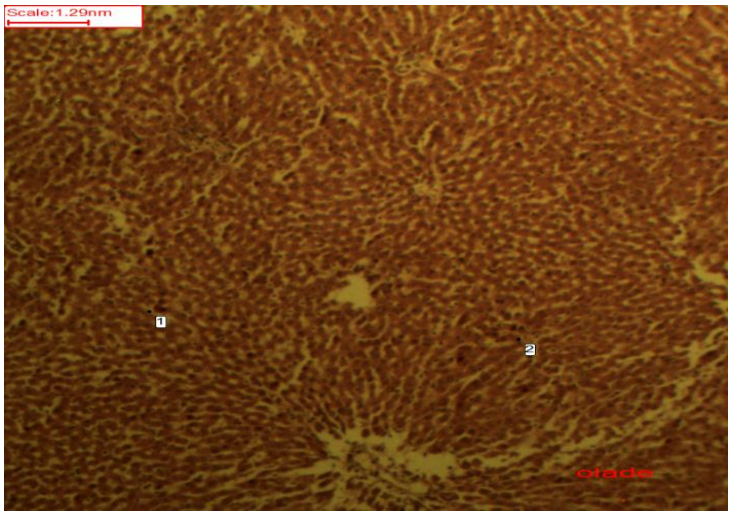

Plate 1: Liver of treated Group B showing mid zonal necrosis (1 and 2) and infiltrating pleomorphic cells.

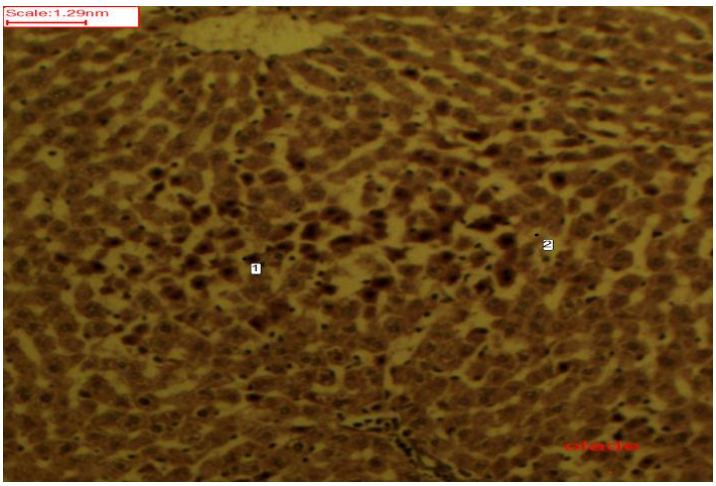

Plate 2: Liver of treated Group C showing necrosis of mid zonal area; 1-Pyknotic hepatocytes with dense disintegrating nuclei. 2-Degenerating hepatocytes without nuclei (stained with H & E).

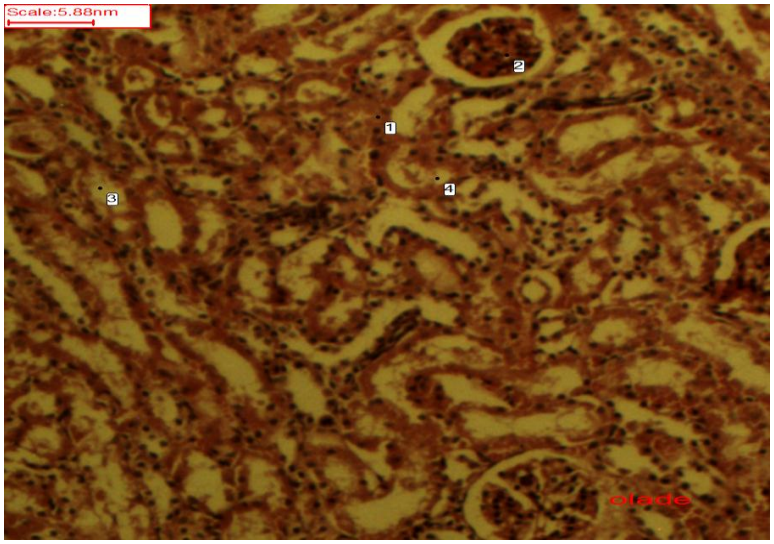

Plate 3: Kidney of treated Group C showing 2-Grossly inflamed glomerulus, engulfed and distended bowman capsule, and 1,3,4-Completely degenerating tubules and the presence of amorphous light staining material occupying the bowman capsule and in some tubules (stained with H & E).

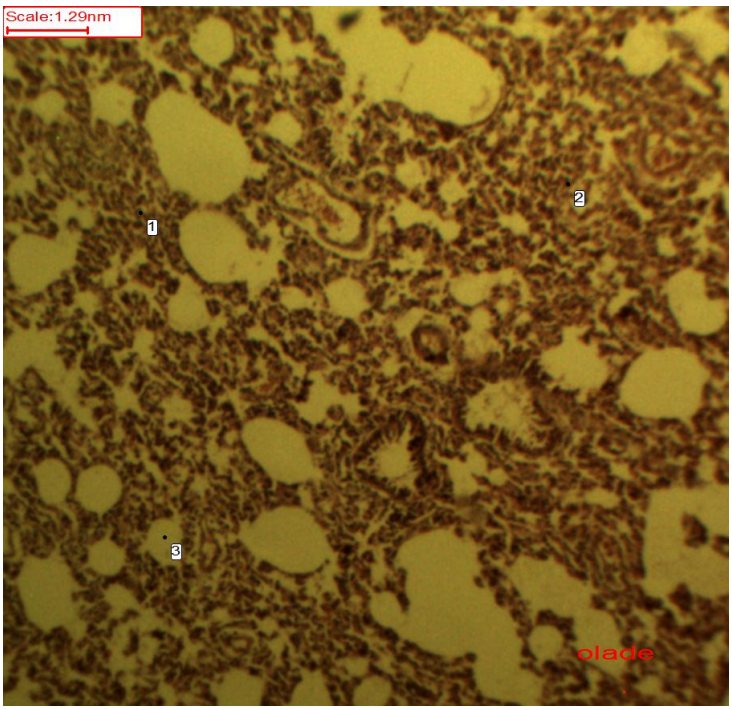

Plate 4: Lung of treated Group C showing hepatisation and collapse of the alveoli septa 1 and 2 (stained with H & E).

DISCUSSION AND CONCLUSION

In this study, the administration of the extract to the Wistar rats does not cause appreciable changes in the haematological parameters, however, the significant increase observed in the

total white blood cell and lymphocytes count could be suggestive of increased immunity due to the plant extract. The immune system is a complex network of cells such as lymphocytes and organs that work together to defend the body against foreign substances. Lymphocytes are one of the main types of immune cells and are divided mainly into B-lymphocytes and T-lymphocytes, while the B-lymphocytes produces antibodies through plasma cells, the T-lymphocytes are programmed to recognise, respond to and to remember the antigens. Many different medicinal plants contain different compounds and have long been used to modulate the humoral and cell-mediated immune responses on Wistar albino rats (Barkatullah *et al.*, 2013) as some herbs are used as antioxidants that detoxify the generated free radicals and stress factors in the body (Steenkamp *et al.*, 2013). Neutrophils in this study are slightly decreased, although the decrease is not significant. The neutrophils represent a multifunctional cell type in innate immunity that contributes to bacterial clearance by recognition, phagocytosis and killing of foreign bodies (Guyton and Hall, 2006; Srikumar *et al.*, 2007), whereas the T and B-lymphocytes are involved and responsible for production of antibodies leading to enhancement of immunity (Guyton and Hall, 2006; Srikumar *et al.*, 2007; Soehnlein *et al.*, 2008). The analysis of the blood serum showed a significant increase in the activities of alanine aminotransferase and aspartate aminotransferase. Elevation of aspartate aminotransferase can be associated with cell necrosis of many tissues. For example, pathology of the skeletal or cardiac muscle and or the hepatic parenchyma allows for the leakage of large amount of this enzyme into the blood (Kaneko, 1980). Alanine aminotransferase on the other hand, is present in liver and other cells and it is particularly useful in measuring hepatic necrosis especially in small animals (Cornelius, 1989). Serum levels of both alanine aminotransferase and aspartate aminotransferase become elevated whenever disease processes affect liver (Johnston, 1999) and thus alanine aminotransferase is used to detect liver diseases and bone disorders (Tolman, 1999). The histopathological studies also showed some changes in the integrity of the liver, kidney and the lungs. It can be therefore concluded that high doses of methanolic extract of root bark of *Afrormosia laxiflora* is toxic to the rats and caution should be taken when using the extract for therapeutic purposes in order to prevent untoward effects.

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