Aqueous Ethanolic Extract of *Mangifera indica* Stem Bark Effect on the Biochemical and Haematological Parameters of Albino Rats

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ABSTRACT

This study was conducted to evaluate the effect of aqueous ethanolic extract of Mangifera indica stem bark on the biochemical and haematological indices of adult albino Wistar rats, so as to determine how safe the extract is in animals. Ten rats were separated into two groups, the first group served as control while the second group was administered 5000 mg/kg body weight of the extract by orogastric intubation for 14 days. The results showed that there was significant elevation (p < 0.05) in the obtained values of white blood cells count, lymphocytes, monocytes, neutrophils, red blood cells and packed cell volume in the treated rats when compared with the control. There was also a significant (p < 0.05) increase in the serum levels of Aspartate transaminase, Albumin and Alanine transaminase in the treated rats when compared with the control. These suggest that the aqueous ethanolic extract of M.indica stem bark might have immunostimulating properties, may also stimulate the haematopoietic system and reduce oxidative damage of red blood cell membranes but may be slowly injurious to hepatic tissues of animals at high doses and prolonged duration of treatment.

Keywords: *Mangifera indica*, Albino rat, Haematological parameters, Serum enzymes,

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INTRODUCTION

Plants have been used for food, medicine, building materials and fuel for many centuries until now [2]. The World Health Organization (WHO) noted that of the 119 plants-derived pharmaceutical drugs, 74 % are used in modern medicine in ways that correlated directly with their traditional uses as Herbal plant medicine by native culture. WHO estimated that about 80 % of the world population presently uses herbal medicine for some aspects of their primary health care needs while plant products also play important roles in the health care system of the remaining 20 %, who mainly reside in developed countries [11]. *Mangifera indica* L. belongs to the family Anacardiaceae; the tree is a native of tropical Asia and is indigenous to the Indian subcontinent, though it is now completely naturalized in many parts of the tropics and subtropics [21]. Other *Mangifera species* include *M*. f*oetida* (Horse Mango) which are grown in more localized areas but *Mangifera indica* - the common mango or Indian Mango - is the only mango species cultivated in many tropical and subtropical regions and its fruits are distributed worldwide. The tree is grown widely in different parts of Africa, and in Nigeria, it is found commonly in Benue and other states of the country. *M indica* is used as medicine to treat several ailments such as asthma, cough, diarrhoea, dysentery, jaundice, pain, malaria, anaemia and diabetes [16, 19, 20].

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Millions of people in various traditional societies including Nigeria have resorted to the use of medicinal plants to treat their ailments. This dependence on natural products has its merits, but care must be taken not to consume harmful plants or high dose of plant extracts that could have deleterious effects on vital organs of the body either in the short term or long term. There are concerns by certain medical personnel that herbal medicines may be harmful to vital organs such as liver and kidneys. However, there seems to be paucity of information on safety evaluations of medicinal plants unlike the therapeutic potentials. Thus, there is need to study the effects of these plants, which have potential for therapeutic benefits, to ascertain their safety in animals using biochemical and haematological indices. Biochemical tests have immense benefits in the diagnosis and monitoring of liver diseases[23], while haematological assays are also useful in early detection of the effects of harmful xenobiotics. The present study was therefore initiated to investigate the effect of administering high dose aqueous ethanolic extract of *Mangifera indica* stem bark on the biochemical and haematological indices of albino Wistar rats, to determine their safety in animals.

MATERIALS AND METHODS

Mangifera indica stem barks were harvested from North Bank area of Makurdi (07^0 $41^{'}$ N, 08^0 $37^{'}$ E), Benue State, Nigeria. The plant was identified and authenticated at the Botany Department of University of Agriculture, Makurdi, Benue State, Nigeria.

Preparation of plant extract
The barks of *M. indica* stem were collected, then cut into smaller pieces and sun-dried for two weeks. The dried barks were pulverized with mortar and pestle, and then further sieved to obtain a coarse powder. The extraction was carried out by a method earlier described [1]. Briefly, the aqueous ethanolic solvent for extraction was prepared by mixing 800 ml of absolute ethanol and 200 ml of distilled water, to obtain 80 % aqueous ethanol. A 100 g coarse powdered stem bark was wrapped with a white piece of cloth, tied and soaked in 500 cm^3 of the 80 % aqueous ethanolic solvent (ratio1: 5) for 48 hrs. The extract /solvent mixture was filtered with Whatman No.1 filter paper. The filtrate was left to dry at room temperature on laboratory bench, to obtain a crude extract.

Experimental animals
Permission was granted by animals' caretakers of Benue state University to obtain some rats for scientific research. Twenty adult male Wistar strain of albino rats, *Rattus norvegicus albinus,* weighing 160 – 240 g, were purchased from the Animal breeding centre, Benue State University, Makurdi, Benue State, Nigeria. They were acclimatized to the experimental room for two weeks at room temperature with 12:12 hr Light: Dark cycle. They were kept in clean and large cages, fed with standard feeds purchased from Vital Feeds, Jos, Plateau State, Nigeria and clean drinking water was provided *ad libitum*. The rat's body weights were determined with a top-loading balance. Ten rats less by one were used for acute toxicity studies while ten rats were used for the main study. The animals were handled in accordance with the guidelines and recommendations of the ethical committee on the use of laboratory animals for research.

Acute toxicity test
The LD$_{50}$ was carried out by the up-and-down procedure [4]. Nine (9) rats were used for this study. Six rats were separated into three groups of 2 rats each. The 3 groups of rats (grp1-3) were administered aqueous ethanolic extract of *M. indica* stem bark orally at 1000, 1500 and 3000 mg/kg body weight respectively, and observed for signs of toxicity like behavioural changes, increased respiratory rate, nervous imbalance and death within 48hrs. Since there were no signs of toxicity or death, another 3 groups (A - C) of one rat per group were given oral dose of the same extract 3000, 4000 and 5000 mg/kg body weight respectively. These rats were also observed for signs of toxicity over 48 hrs and the LD$_{50}$ was determined.

Experimental design and administration of extract
The rats were randomly allocated into two groups of 5 animals each. Rats in group A were untreated with extract but given equivalent volume of distilled water per kg body weight, and serve as control while those in group B were treated with aqueous ethanolic extract of *M. indica* stem bark at a dose of 5000 mg/kg body weight of the extract re-suspended in distilled water. The administration of extract is by daily orogastric intubation for a period of fourteen days.

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Blood collection
At the end of treatment with extract, rats were anaesthesized with chloroform and blood was collected from them by cardiac puncture. About 1ml of blood was dispensed into specimen bottles containing the anti-coagulant sodium ethylene diamine tetra-acetic Acid (EDTA), while another 1 ml was dispensed into clean Bijou bottles without anti-coagulant and left to clot, to be used for biochemical studies.

Haematological assays
The whole blood with anticoagulant was used for assay of the haematological parameters using the XF 9080 Animal Haematology Auto-analyzer (Hitachi, Germany). Parameters determined are packed cell volume (PCV), Red blood cells (RBC) count, White blood cells (WBC) count, mean corpuscular Haemoglobin concentration (MCHC), mean corpuscular volume (MCV) Lymphocytes, Monocytes and Neutrophils, using standard assay kits (Roche diagnostics Ltd, United Kingdom).

Biochemical assays
Blood samples in the tubes without anticoagulant were allowed to clot at room temperature and then spinned with centrifuge at 2000 rpm for 20 minutes. The sera were aspirated with clean and dry pipettes; and the biochemical parameters were determined with an automated analyzer (Hitachi 902, Germany). The parameters are Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Albumin, Total protein, Triglycerides (TG), Total cholesterol, Creatinine, Total bilirubin, Direct bilirubin, High density lipoprotein (HDL) cholesterol and Low density lipoprotein (LDL) cholesterol. The assay was conducted using standard assay kits (Roche diagnostics Ltd, United Kingdom).

Statistical analysis
All data obtained were subjected to statistical analysis using Student's t-test using Statistical Package for Social Sciences (SPSS for windows, version 12.0). Data were expressed as mean ± standard error of mean (SEM). Values of $p < 0.05$ were considered significant.

RESULTS

The minimum lethal dose (LD_{50}) of the *M. indica* extract was estimated as > 5000 mg/kg body weight. Thus, the acute toxicity study showed the extract to be of low toxicity at a dose of 5000 mg/kg body weight, as there were no clinical signs of toxicity nor any death after 48 hours post-administration.

Haematological assays
The oral dose of 5000 mg/kg body weight of *M. indica* stem bark extract produced a significant ($p < 0.05$) elevation in the values of PCV, WBC counts, RBC counts, Lymphocytes, Monocytes, Neutrophils, MCHC; but no significant difference ($p > 0.05$) in the values of MCV (Table1).

Biochemical assays
The biochemical evaluations showed that oral administration of the extract at the given dose produced significant($p < 0.05$) increase in Aspartate aminotransferase, Alanine aminotransferase, and albumin, while there was no significant ($p > 0.05$) difference in the levels of bilirubin, total protein, creatinine, all forms of cholesterols, alkaline phosphatase and triglycerides (Table 2).

Table1: Effect of *M. indica* stem bark extract on Heamatological Parameters of Rats

Parameters	Group A (Control)	Group B (5000mg/kg)
White blood cells (x 10^3/µl)	3.38± 0.95	15.45± 0.11*
Lymphocytes (%)	61.53± 0.18	68.16± 1.53*
Monocytes (%)	4.70± 0.19	6.14± 0.52*
Neutrophils (%)	21.02±1.38	25.12±1.56*
Red blood cells (x 10^6/µl)	3.41± 0.33	6.08± 0.70*
Packed cell volume (%)	40.7± 0.08	48.1± 0.13*
Mean corpuscular volume (fl)	170.6± 2.8	167.6± 5.99
Mean corpuscular haemoglobin concentration (g/dl)	27.20± 1.94	53.4± 1.54 *

Values represent the Means ± SEM, n = 5. The values with asterisk are significantly different from the control at p < 0.05.

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Table 2: Effect of *M. indica* extracts on the Biochemical Parameters of Rats

Parameter	Group A (control)	Group B (5000mglkg)
Alanine aminotransferase (IU/l)	194.8 ± 26.77	$279.5 \pm 71.35^*$
Aspartate transaminase (IU/l)	242.3 ± 37.07	$306.9 \pm 48.18^*$
Alkaline phosphatase (IU/l)	358.0 ± 39.32	376.0 ± 38.41
Total protein (g/l)	71.88 ± 3.59	77.0 ± 2.09
Total bilirubin (mmol/l)	13.3 ± 0.12	12.3 ± 0.12
Direct bilirubin (mmol/l)	1.82 ± 0.01	1.62 ± 0.13
Albumin (g/l)	33.4 ± 1.97	$38.78 \pm 1.34^*$
Creatinine (g/l)	56.26 ± 5.19	56.55 ± 2.49
Total cholesterol (mmol/l)	1.34 ± 0.18	1.74 ± 0.17
Triglycerides (mmol/l)	1.14 ± 0.70	1.28 ± 0.54
High density lipoprotein cholesterol (mmol/l)	1.0 ± 0.09	1.26 ± 0.12
Low density lipoprotein cholesterol (mmol/L)	2.98 ± 0.6	2.72 ± 0.08

Values represent the Means $\pm$ SEM, n = 5. The values with asterisk are significantly different from the control at p < 0.05.

DISCUSSION

The oral minimum lethal dose (LD_{50}) of the aqueous ethanolic extract of *M. indica* stem bark estimated as > 5000 suggests that the extract may have low toxicity. It was earlier established that any substance with LD_{50} estimate greater than 2000 mg/kg body weight by oral route may be considered of low toxicity and safe in humans [3, 5]. Earlier study [24] showed no mortality when a limit dose of 2000 mg/kg body weight of an extract was administered to animals orally and indicated that the extract has low acute toxicity.

Red blood cells are extremely susceptible to lipid peroxidation, since they are rich in unsaturated membrane lipids, have rich supply of oxygen and transition metal catalyst. Lipid peroxidation occurs as a result of the reaction between free radicals and membrane lipids and is considered as an important feature of cellular injury[25]. The observed significant increase in PCV, RBC counts, and MCHC of the test rats may suggest that the extract of *M. indica* stem bark has haematopoietic property and might enhance erythropoiesis of animals and may increase resistance to oxidative damage to red blood cells membranes. This is in agreement with previous studies [18] which reported that *M. indica* stem bark extract has potential for boosting the haematopoietic system of rats, and it was also earlier reported that *M. indica* stem bark extract has antianaemic properties, so might improve erythropoiesis and probably reduce oxidative damage to RBC membranes [19], thereby preventing exposure of the erythrocytes to destruction by macrophages [15], in anaemic rabbits earlier induced by phenylhydrazine hydrochloride. The antianaemic property of *M. indica* stem bark extract was attributed to the presence of some phytochemicals which act as antioxidants in animals [19]. The significant increase in WBC and the differential leukocytes counts in the test animals suggests that the extract may have immunological properties, by stimulating increased production of white blood cells, thereby boosting the defence system of the animals. This is in agreement with earlier findings [10] that *M. indica* extract might have immunostimulating properties, which may account for the use of the plant for medicinal purposes by traditional medical practitioners. Herbal extracts have potential application as immunostimulants and could act against a broad spectrum of pathogenic microorganisms. The effect is dose-dependent and there is the tendency for overdose to occur, therefore dosage optimization is strongly recommended[26].

Alanine aminotransferase (ALT) and Aspartate aminotransferase(AST) have been established as markers of hepatocellular injury while Alkaline phosphatase(ALP) is a marker of cholestasis [1, 23]. The significant elevation in the activities of AST and ALT in the serum of test animals suggests that aqueous ethanolic extract of *M. indica* stem bark might induce hepatocellular injury in animals. These increases of ALT and AST levels in serum may be due to leakage from hepatocytes through peroxidative damage of their membranes [12] leading to increased membrane permeability. Findings have shown that persistent elevation of serum ALT, AST and ALP are reliable markers for alcoholic hepatotoxicity [7, 9]. These enzymes are usually raised in acute hepatotoxicity or mild hepatocellular injury, but tend to decrease with prolonged intoxication, as a result of severe damage to the liver [8, 14]. Earlier investigators reported an increase in serum ALT activity in methimazole-induced hepatotoxicity in mice [17] while other findings revealed an increase in ALT activity in oral acetaminophen-induced hepatotoxicity in rats [22], providing a biochemical evidence of significant liver damage. The oral administration of 100mg aqueous extract of *M. indica* stem bark for 14 days causes a significant increase in AST activity [13]. Serum albumin and total protein are some of the markers of liver dysfunction while albumin transports bilirubin and other substances in blood [23]. The significant increase in the level of albumin may indicate that the synthetic function of the liver has

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not been significantly affected yet and also suggest that free albumin is elevated due to the decreased level of bilirubin in the test animals. The bilirubin formed from breakdown of red blood cells in the reticulo endothelial cells are transported in plasma bound to albumin [23], so the decrease in bilirubin level, as a result of reduction in the natural oxidative break down of red blood cells, may account for the observed increase in albumin, as less albumin was bound in the treated rats. It therefore seems that the *M. indica* stem bark extract might provide resistance to oxidative break down of red blood cells membranes and may be considered safe only at lower doses but at higher doses might be injurious to the liver hepatocytes. The   non significant difference in the levels of Alkaline phosphatase, Bilirubin, Total protein, Creatinine, Cholesterols and Triglycerides suggests that this extract, even though might increase or decrease these biochemical parameters in animals, but may not interfere significantly with the metabolism of these biochemical parameters within the duration of the study.

CONCLUSION

This study therefore showed that aqueous ethanolic extract of *Mangifera indica* stem bark may increase the rate of erythropoiesis, slow down the natural process of oxidative breakdown of RBCs and may also promote the immune-stimulatory activities of WBC, but might be slowly injurious to the hepatocytes of animals at higher doses and long treatment regime. Therefore, those who use the crude extract for treatment of diseases should exercise caution, to avoid high doses and prolonged treatment, which might have deleterious effect on vital animal tissues.

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