



ANTIBACTERIAL AND TOXICOLOGICAL EFFECTS OF THE AQUEOUS EXTRACT OF MANGIFERA INDICA STEM BARK ON ALBINO RATS

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ABSTRACT

Mangifera indica (MI) otherwise known as mango is popular for its sweet succulent fruits eaten as food. The leaves and the stem bark are used as herbal medicine. Many studies have been conducted on the phytochemical constituents and many work done to scientifically prove their efficacy as antibacterial and antifungal agents. However it appears that work on its effectiveness as an antityphoid agent and toxicity study of aqueous MI stem bark extract are still lacking. In this study investigation on the antibacterial activities was done on salmonella typhi and six other bacteria. Acute toxicity studies was also carried out using albino rats and fixed doses of the extract as follows; 10mg/kg, 100mg/kg, 1000mg/kg, 2900mg/kg and 5000mg/kg body weight of the animals. The extract was found to be active against all the test bacteria except *streptococcus faecalis*. There was no sign of toxicity and no death recorded even at 5000mg/kg. The single oral dose did not produce any statistical significant changes at $p < 0.05$ in haemological parameters like the HB and HCT, the biochemical parameters like the AST and ALT when the control group was compared to the test group. The histological examination also compare well with the control.

KEY WORDS: Antibacterial, Bark, Mango and Toxicity.

INTRODUCTION

Medicinal plants have been sources of a number of important compounds which have been discovered during the last century. In the light of their established therapeutic efficacy, the pharmaceutical industries are using crude extracts of medicinal plants for manufacturing drugs [1].

Research conducted on medicinal plants have served the dual purposes of bringing up new therapeutic agents and providing useful leads for studies directed towards the synthesis of drugs on the basis of the chemical structures of the natural products. Modern pharmaceutical industries still rely to some extent on the bioactive principle, obtained from plants. For example, the anticancer agent, taxol, Isolated from the pacific yew, *taxus brevifolia* [2] and the antimalarial agent artemisinin obtained from the Chinese herb *Artemisia annua* [3].

80% of the world population still depends on herbal medicine as their main source of medicinal therapy [4]. Today many scientists and medical experts around the world are emphasizing the value of herbal remedies for health. Only a small fraction of earth's plants have been investigated scientifically leaving an enormous unexplored potential. From the foregoing, it is apparent that more organized efforts are required for bioassay directed isolation studies of natural products from medicinal plants.

Mango tree also known as *Mangifera indica* is a large ever green tree native to the tropics and subtropics [5]. Mango is the most economically important plant in the family of anacardiaceae with India being the largest producer [6]. In Nigeria (West Africa) *M. indica* is usually commonly used as herbal preparations in the treatment of toothache, gastrointestinal disorders, dysentery, diarrhea, gastrointestinal tract infections, respiratory and urinary tract infections, sore gums and sore throats.

In separate studies Agoha [7] and Madunagu [8] established that *Mangifera indica* is used against asthma, cough, diarrhea, dysentery, jaundice pains and malaria. In all the region of *Mangifera indica* distribution, one of the main organs used is the bark. Based on ethnopharmacological knowledge, a standardized aqueous extract of the plant's stem bark possessing antioxidant, anti-inflammatory and immunomodulatory properties has been developed in Cuba. This extract is proposed as both nutritional supplement (antioxidant) and an anti-inflammatory, analgesic and immunomodulatory treatment to prevent disease progress or increase the patient's quality of life in gastric and dermatological disorders, AIDS, cancer and asthma [9].

Mangiferin found in the stem bark of mango tree is an astringent and is employed against rheumatism and diphtheria in India. Other pharmacological activities of mangiferin are as follows: Antioxidant [10], radio protective effect [11,12], immunomodulatory [13], antiallergic activity, [14], anti-inflammatory activity [15], antitumor activity [16,17,18], Lipolytic [19], Antibone resorption [20], Antimicrobial [21,22,23], antibacterial, antifungal [24] and antiparasitic activities [25].

Many bioactive compounds abound in mango pulp, peels, seeds, leaves, flowers and stem bark due to their antioxidative, antimicrobial and other health promoting properties that make consumption of mangoes and its derived product a healthy habit [9, 26]. The chemical constituents of the *Mangifera indica* bark include protocatechic acid, catechin, mangiferin, alanine, glycine, γ -amino butyric acid, kinic acid, shikimic acid and tetracyclic triterpenoids [27]. Other compounds isolated from *M. indica* extract are terpenoidal saponins, polygalacturonase, fructose -1-6-diphosphatase, triterpenoid, tetracyclic triterpenoids, pentacyclic triterpenoid and 2-hydroxy mangiferonic acid [28]. The

main constituent of the leaf extract is citric acid, although glycolic, oxalic, malic aspartic acid, glutamic acid, alanine, glycerine, serine, amino butyric acid and tartaric acid are found in the extract [29]. *Mangifera indica* contains alkaloids and glycosides which are of great importance pharmacologically [8, 30].

Also El Mahmood [31] found that the crude extract of mango stem bark has alkaloids, phenols, tannins, saponins and cardiac glycosides. The aqueous leaf extract of M.I is rich in polyphenols amongst which is mangiferin which has been extensively studied by many authors [32]. The natural C-glucoside xanthine mangiferin has been reported in various parts of *Mangifera indica*: Leaves [33], fruits [34] and stem bark [35].

Typhoid fever is an acute system infection caused by the bacterium salmonella enterica serovar typhi [36].

Salmonella enterica serovars paratyphi A, B & C cause the clinically similar condition, paratyphoid fever. Typhoid and paratyphoid fevers are collectively referred to as enteric fevers. In most endemic areas, approximately 90% of enteric fever is typhoid [37]. The organism was variously known as Bacillus typhosus, Erbethella typhosa and salmonella typhi [37]. The WHO [38] estimated that one third of the world wide population was infected with the bacterium. In 2004 researchers [39] put the figure at approximately 22 million cases of typhoid with at least 200,000 deaths. Typhoid is more severe in the developing countries because of poor hygiene, indiscriminate use of antibiotics and a rapid rise in multidrug resistance [40]. In 1948 chloramphenicol became the standard antibiotic for treating typhoid [41]. Although resistance emerged within two years after its introduction, it was not until 1972 that chloramphenicol-resistant typhoid fever became a major problem [42]. Toward the end of the 1980s and the 1990s, *S. enteric* serotype typhi developed resistance simultaneously to all the drugs that were then used as first-line treatment (chloramphenicol, trimethoprim, sulfamethoxazole, and ampicillin) [42]. Following this problem it became imperative to look for other avenues to treat and manage typhoid fever.

In Nigeria a survey of forest plants used in the traditional treatment of typhoid fever was conducted [43] and *Mangifera indica* (mango) stem bark was found to be among the plants that are very effective when cooked with water. Some of which is used for bathing the patient while some is drunk by the patient. This finding and the fact that there appears to be little knowledge on its toxicological side effects were what informed our interest to scientifically look into the antibacterial and toxicological effect of this plant on albino rats.

MATERIALS AND METHOD

Chemicals and Reagents

Chloroform and other solvents used were obtained from Zayo Chemicals and were of ANALAR grade. All laboratory reagents as far as possible were of ANALAR grade.

Equipment

Blood cell counter BC-2800 Vet, Flame photometre, Spectrophotometre (Optima sp-300), Shandom automatic tissue processor, hot plate, Microscope, Mettler weighing balance, Ohaus Harvard trip balance, Hot air oven, Rotary evaporator.

Plant Material

The stem bark of *Mangifera indica* trees were freshly collected from Vom in Jos south L.G.A, Plateau state Nigeria and were identified at the Federal Department of Forestry Jos.

Experimental Animals

Albino rats of both sexes weighing 91g-180g were purchased from the small animal house of federal college of Veterinary and Medical Laboratory Technology, National Veterinary Research Institute, Vom and were maintained on pelleted feeds obtained from Dagwom farms N.V.R.I, Vom.

Hot Water Extraction of the Crude Plants' Materials

The barks of the above mentioned plants were washed with clean water to get rid of dust and dirt and were air dried to get rid of the water droplets. They were oven dried at 60°C for seven days. The dried plant materials were pulverized into course powder in a mortar with a pestle. They were ground into fine powder with an electric blender. 100g of the plant sample was extracted in hot water. The solutions were filtered and the filtrate evaporated off under reduced pressure in a rotary evaporator to obtain the crude extract.

Phytochemical Screening of the Crude Plants' Extracts

The extracts were subjected to phytochemical screening to detect the presence of the following secondary metabolites; Resins, Alkaloids, Saponins, Tannins, Glycosides and Flavonoids following standard procedures [44].

Test for resins

0.5g of the extract was added to 5ml of boiling ethanol. This was filtered through Whatman No. 1 filter paper and the filtrate diluted with 4ml of 1% aqueous HCL. The formation of a heavy resinous precipitate indicated the presence of resins.

Test for alkaloids

0.5g of the extract was stirred with 5ml of 1% aqueous HCL on a steam bath. This was filtered and 1ml of the filtrate treated with a few drops of Dragendorffs reagent and a second 1ml portion treated similarly with Wagner's reagent. The formation of precipitates was an indication of the presence of alkaloids.

Test for saponin

0.5g of the extract was shaken with water in a test tube. Frothing (foaming) which persists on warming was taken as preliminary evidence for the presence of saponins

Test for tannin

0.5g of the extract was stirred with 10ml of distilled water. This was filtered and a few millimeters of 5% ferric chloride added to the filtrate. A deep green coloration showed the presence of tannin. A second portion of the filtrate was treated with a few millimeters of iodine solution. A faint bluish coloration confirmed the presence of tannin.

Test for glycosides

0.5g of the extract was stirred with 10ml of boiling distilled water. This was filtered and 2ml of the filtrate hydrolyzed with a few drops conc. HCL and the solution was added to 2ml of Benedict's qualitative reagent and boiled. A reddish-brown precipitate showed the presence of glycosides.

Test for flavonoids

0.5g of the extract was dissolved in 2ml dilute NaOH solution. A few drops of conc. H₂SO₄ were then added. The presence of flavonoids was indicated as the solution becomes colourless.

RECONSTITUTION OF THE CRUDE PLANTS' EXTRACTS

The crude extract was reconstituted in sterile distilled water to get the following concentrations: 200mg/ml, 400mg/ml, 600mg/ml, 800mg/ml and 1000mg/ml.

SCREENING OF ANTIBACTERIAL ACTIVITIES

Punch Hole Diffusion Method

An inoculum size of 10⁸ cfu/ml of the clinical isolates of the different gram-positive and gram-negative bacteria was prepared according to the method of Bauer *et al.* [45]. Five gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella* and *Salmonella typhi*. Also *Staphylococcus aureus* and *Streptococcus faecalis* (gram-positive bacteria) were used.

The nutrient agar plates were inoculated by flooding with the inoculum of the different isolates. The plates were dried in an incubator at 37°C and seven holes were bored on each of the seeded plates, using a 6mm diameter sterile cork borer. A drop of molten nutrient agar was put into each hole to seal off from the bottom of the plate. The different concentrations of the crude extracts used to fill the holes corresponding to their respective labels, avoiding overflowing. Sterile distilled water and ciprofloxacin (10 mg/ml) were used as negative and positive controls. All the plates were incubated aerobically at 37°C for 24hrs. Diameters of the zones of inhibition were measured in millimeters (mm) and recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using freshly prepared nutrient broth. 9ml of the freshly prepared nutrient broth was added to each of the sterile test tubes labeled 2-6. 1ml of 200mg/ml, 400mg/ml, 600mg/ml, 800mg/ml and 1000mg/ml of the various plants' crude extract were added to tubes 2-6 respectively, tube 1 is the positive control containing 10mls of nutrient broth. Using a sterile pipette, 50µl of the test organism was added to each of the six tubes. The contents of the tubes were thoroughly mixed and incubated at 37°C for 24hrs. The tubes were then inspected for turbidity. Turbidity shows bacterial growth. The minimum concentration without bacterial growth (i.e. without turbidity) is the minimum inhibitory concentration. The results are shown in tables

Determination of Minimum Bactericidal Concentration

Minimum bactericidal concentrations (MBCs) of the crude extract preparations were determined by agar diffusion method [46]. 10µl suspension from each negative tube in the MIC assays and from the positive growth control tubes were incubated aerobically at 37°C for 24hrs. MBCs were defined as the lowest concentration of the extract that produced negative subcultures. The result is shown in the table

ACUTE TOXICITY STUDIES

Crude Extract

The hot water extracts of the aforementioned plants were prepared as earlier described.

Acute Toxicity Determination

The acute toxicity studies were carried out as follows according to Lorke [47] with slight modification. The acute toxicity was performed in two stages.

STAGE 1:

In this initial investigation the range of the doses producing the toxic effects is established.

STAGE 2:

Based on the results from stage one further high doses are administered to calculate the LD₅₀. The LD₅₀ is calculated as the geometric mean of the doses for which 0/1 and 1/1 animals died. For example, at 2900mg/kg 0% animal died and at 5000mg/kg 100% of the animals died. The LD₅₀ is the geometric mean of 2900 and 5000 which is 3800mg/kg.

Lorke's method is based on the assumption that the chemical substances under investigation are completely unknown and the investigation is to be carried out using a minimum number of experimental animals. The determination of the appropriate dosage range of the acute toxicity is achieved by giving widely differing doses to the animals. E.g.

10mg/kg, 100mg/kg and 1000mg/kg body weight of the animals. The results show whether the chemical substance is very toxic, toxic, less toxic, slightly toxic or not toxic. A total of 16 animals were used for the plant extract. Nine animals for the 1st stage, 3 animals as the control, and four animals for the 2nd stage.

Acute Toxicity Methodology

The animals were kept in the experimental house for five days for adaptation. Food and water was given *ad libitum*. The animals were individually weighed using Ohaus Harvard trip balance. The animals were also given numbers using the conventional method of numbering animals. Food and water were withdrawn from them the night before the commencement of the experiment.

1st STAGE

The animals were divided into 3 groups of 3 animals per group. The 4th group has 3 animals and serves as the control. Group 1 was given the plant extract of 10mg/kg body weight of the animal, group 2 was given 100mg/kg and group 3 was given 1000mg/kg. Group 4 was given a volume of distilled water in commensuration with their body weights. The animals were observed frequently on the day of treatment until the office closing hour of 4pm. The animals were monitored to see if the plant extracts produced the effect of dermal or oral irritations as would be manifested as rubbing of mouth, stretching of limbs and scratching. Other symptoms of toxicity like difficulty in breathing, disinclination to move, or eat, sleepiness and death were observed for. On the first day of the experiment, the animals were monitored hourly and subsequently (for 14 days) the animals were monitored on daily basis. Also the animals were weighed before the commencement of the experiment and on the day of the termination of the experiment. Weight gain or loss was recorded. After 14 days the animals were anaesthetized with chloroform and the blood collected through direct heart puncture. 2mls were added into E.D.T.A anticoagulant bottles and 4mls into plain centrifuge tubes. The blood collected into anticoagulant bottles were used for haematological analysis while the serum was collected after centrifugation at 3000 r.p.m for 5 minutes for biochemical analysis. The animals were thereafter sacrificed and autopsied and examined macroscopically for any pathological changes. Samples of the liver, and kidneys were also collected into 10% formalin for histological studies.

2nd STAGE

The animals were divided into 4 groups of one animal per group. Group 1 was given a dose of 1600mg/kg body weight, group 2 was given 2900mg/kg body weight, group 3 was given 5000mg/kg and group 4 distilled water in a volume calculated from the body weight of the animal. Observations of the animals were carried out as in the 1st stage. Also the entire steps of the 1st stage were repeated until the experiment was terminated on the 14th day and LD₅₀ calculated.

METHOD FOR HAEMATOLOGICAL ANALYSIS

The blood collected (in EDTA as anticoagulant) was analyzed for total white blood cells (WBC), lymphocytes, monocytes, granulocytes, red blood cells (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration (MCHC), red cell distribution width, platelets, mean platelet volume, platelet distribution width, and packed platelet volume using fully automated blood cell counter BC-2800 Vet.

METHODS FOR BIOCHEMICAL ANALYSES

The serum collected from the clotted blood was analyzed using a flame photometer and spectrophotometer at the appropriate wavelengths using the appropriate reagents. Tests were performed for the electrolytes; sodium, potassium, and chloride. Also bicarbonate, urea, creatinine, total protein, albumin, total bilirubin, conjugated bilirubin, alkaline phosphatase (ALK), aspartate amino transferase (AST), and alanine amino transferase (ALT) were performed.

METHOD FOR HISTOLOGICAL ANALYSIS

The samples of liver kidney and heart collected separately into 10% formalin were processed using Shandon automatic tissue processor. The tissues sectioned into 5 micrometer sizes were attached to glass slides by water bath method. The section was allowed to dry on a hot plate. Harris haematoxylin and eosin was used to demonstrate the general tissue structure under a microscope.

RESULTS

ANTIBACTERIAL STUDIES

The antibacterial study shows that the aqueous extract of mango stem bark has antibiotic activity. Table 1 shows the zones of inhibition of the extract at various concentrations against *S.typhi* and six other Gram positive and negative bacteria. It shows that the antibiotic activity increases with increase in concentration of the extract. The extract does not have any effect on *streptococcus faecalis*. Table 2 shows the result of the minimum inhibitory concentration and for this extract it was found to be 600mg/ml. The maximum bactericidal concentration is also 600mg/ml (Table 3). This shows that at 600mg/ml the extract totally inhibited the growth of *S. typhi*. The high MIC and MBC are also indications that this plant extract is more effective at high concentrations.

Table 1: Antibacterial activities of hot aqueous extract of *Mangifera indica* (mango) stem bark.
ZONES OF INHIBITION OF THE VARIOUS BACTERIA (mm)

Concentration of extract (mg/ml)	200	400	600	800	1000	-ve	+ve
<i>Salmonella typhi</i>	-	12	12	13	16	-	27
<i>Shigella</i>	8	9	9	9	10	-	26
<i>Proteus vulgaris</i>	11	14	16	16	16	-	26
<i>Staphylococcus aureus</i>	12	12	16	16	16	-	25
<i>Streptococcus faecalis</i>	-	-	-	-	-	-	20
<i>Pseudomonas aeruginosa</i>	9	10	10	11	11	-	30
<i>Escherichia coli</i>	8	9	10	10	10	-	28

KEY:

- mm = millimeters
 mg/ml = milligram per milliliter
 -ve = negative control
 +ve = positive control (10mg/ml ciprofloxacin)
 - = no zone of inhibition

Table 2 Minimum inhibitory concentration (MIC)

1:10 dilutions of the various concentration of crude extract of mango barks against *Salmonella typhi*

No of tubes	1	2	3	4	5	6
Concentrations (mg/ml)		200	400	600	800	1000
Nutrient broth (ml)	10	9	9	9	9	9
Extract (ml)	-	1	1	1	1	1
<i>Salmonella typhi</i> (µl)	50	50	50	50	50	50
Turbidity	+	+	+	-	-	-

KEY:

- = no turbidity (no bacterial growth)
 + = presence of turbidity (presence of bacterial growth)

Table 3 Maximum bactericidal concentration (MBC)

Concentration mg/ml	600	800
Bacterial growth	-	-

KEY:

- = no bacterial growth

Phytochemical screening of aqueous extract of mangifera indica stem bark

The result of the phytochemical screening (Table 4) shows that the extract is rich in flavonoids and contains resins but lack alkaloids, saponins, tannins and glycosides

Table 4

	<i>Mangifera indica</i>
Resin	+
Alkaloids	-
Saponins	-
Tannins	-
Glycosides	-
Flavonoids	++

Toxicological Studies

The toxicological studies on the extract show that the plant extract is not toxic to the animals used as they did not die during the course of the experiment but rather increased in weight (Table 5). The haematological parameters, biochemical parameters and histological tissue sections of the liver and kidney of the animals fed with extract compared well with that of the control animals (Table 6.Figures 1 and 2).

Table 5 Effects of the plants' extracts at different doses on rats' weights and the rats' survival rate.

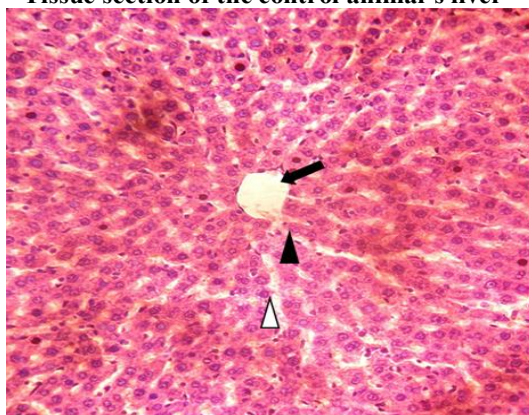
PLANT	DOSE(mg/kg)	WT GAIN(g)	SURVIVAL
MANGO	10	17.53 ± 3.4	100
	100	18.40 ± 3.2	100
	1000	22.37 ± 6.8	100
CONTROL	-	17.60 ± 1.9	100

Table 6. Effect of Mango Extract on Haematological, Liver and Kidney Function of Rats

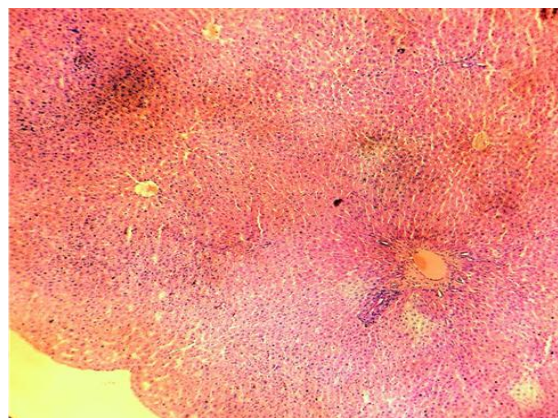
Parameter	Control	Mango (10mg/kg)	Mango (100mg/kg)	Mango (1000mg/kg)	P	
	$\bar{X} \pm \text{SEM}$	$\bar{X} \pm \text{SEM}$	$\bar{X} \pm \text{SEM}$	$\bar{X} \pm \text{SEM}$		
Haematological	Hb (g/dl)	17.40±0.99	17.67±0.71	17.37±0.32	15.10±0.91	0.145
	PCV (%)	43.73±4.62	50.77±1.13	45.70±2.03	44.03±2.44	0.340
	RBC($\times 10^{12}/L$)	8.49±0.27	8.76±0.31	7.65±0.24	8.11±0.38	0.132
	MCHC(g/dl)	356.00±15.87	334.67±3.28	<u>380.00±10.26</u>	340.00±0.00	0.039
	WBC($\times 10^9/L$)	8.15±0.37	7.58±0.08	8.07±0.31	8.92±0.51	0.137
	Platelet ($\times 10^9/L$)	434.00±107.70	536.67±23.60	343.00±86.97	341.00±144.60	0.506
	Granulocyte (%)	87.90±0.55	88.17±0.79	88.70±0.21	87.77±0.61	0.686
	Lymphocyte (%)	9.40±0.49	9.23±0.74	8.73±0.23	9.50±4.52	0.745
	Monocyte (%)	2.70±0.12	2.57±0.03	2.57±0.03	2.73±0.09	0.334
	Liver	Albumin (g/dl)	36.00±2.08	34.00±0.57	32.50±1.44	35.00±2.89
Total Bilirubin ($\mu\text{mol/L}$)		10.07±0.07	10.00±0.00	10.00±0.00	10.00±0.00	0.441
Conj Bilirubin ($\mu\text{mol/L}$)		5.03±0.03	5.00±0.00	5.00±0.00	5.00±0.00	0.441
ALP(iu/L)		82.00±1.15	84.67±2.33	83.00±1.53	83.00±3.00	0.845
AST(iu/L)		15.17±0.23	12.93±1.09	14.23±0.61	14.33±0.18	0.181
ALT(iu/L)		11.17±0.62	11.53±1.43	8.50±0.12	11.77±0.64	0.083
Kidney	Sodium (mmol/L)	4.33±0.33	4.30±0.06	4.40±0.00	4.43±0.09	0.366
	Potassium (mmol/L)	104.00±1.15	103.33±0.67	104.00±0.00	102.67±1.76	0.802
	Chloride (mmol/L)	23.00±1.00	22.33±0.88	22.00±1.15	21.33±0.88	0.696
	Bicarbonate (mmol/L)	5.37±0.23	5.26±0.12	5.05±0.03	<u>5.87±0.20</u>	0.044
	Creatinine (mmol/L)	81.33±1.76	76.33±2.19	<u>87.33±0.88</u>	<u>86.00±1.15</u>	0.004
	Total Protein (g/dL)	71.00±0.58	71.67±1.45	69.50±0.29	70.00±1.15	0.447

P < 0.05 is considered significant

Tissue section of the control animal's liver

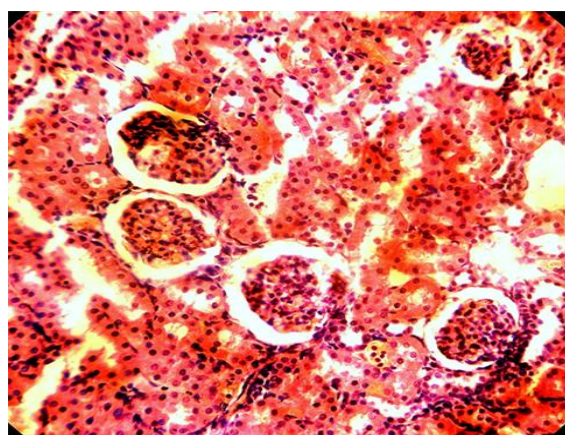


Micrograph of Liver tissue section showing normal appearing hepatic lobule with a centrilobular vein (Black Arrow) from which radiates chords of hepatocytes, in-between which are the hepatic sinusoids (White arrowhead). Occasional Kupfer cells (Black arrowhead) are seen. Haematoxylin and Eosin stain (x40)

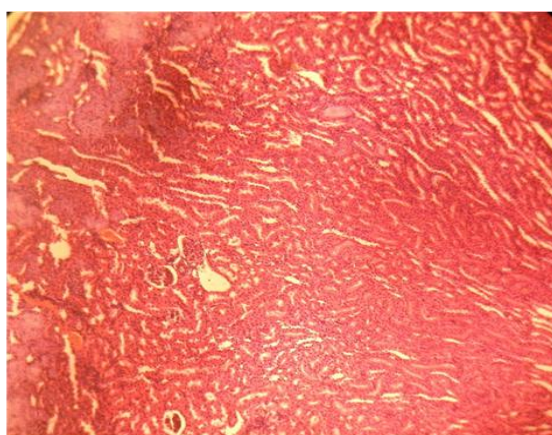


Low power magnification of Liver tissue section showing a preserved hepatic lobular architecture. Periportal inflammatory infiltrates can be observed at the lower right portion. Haematoxylin and Eosin (X 10)

Figure 1 Micrograph of Liver Tissue



Photomicrograph of Control Kidney Section showing normal appearing renal tissue. H and E (Original Magnification X40)



Photomicrograph of Control Kidney Section showing normal appearing renal tissue. H and E (Original Magnification X10)

Figure 2 Micrograph of Kidney Tissue

DISCUSSION AND CONCLUSION

The use of medicinal plants in the treatment of diseases is very common. In Nigeria, rural and urban communities use medicinal plants as food and traditional medicine. *Mangifera indica* (MI) stem bark extract is used traditionally by some population as remedy for gastrointestinal tract problems especially against typhoid fever. Some of these usages have been studied in vitro but there appears to be little systematic research on its antityphoid efficacy and on its toxic effects in vivo. The MI stem bark has antibacterial activities against the various test bacteria investigated (table 1). The antibacterial activity is dose dependent. The optimum activity is at higher dose. The extract is active against all the Gram negative bacteria tested. On the other hand, of the two Gram positive bacteria studied, it is active against *staphylococcus aureus* but fail to act against *streptococcus faecalis*. The streptococcus was resistant to the extract at all the doses used. This could be attributed to the cell envelope differences of the bacteria [50]. Antimicrobial agents make contact with the cell envelope first and the structural difference plays a key role in the susceptibility. Similar results were recorded by Masibo and He [32] who studied the antibacterial effect of aqueous leaf extract of M.I and found that it has a weak antimicrobial activity against both Gram positive and Gram negative bacteria and that lactic acid bacilli (Gram positive and “safe” bacteria) exhibited resistant to the M.I leaf extract. Also El-Mahmood [31] in a separate investigation stated that the aqueous stem bark extract of M.I is active against both Gram positive and negative bacteria tested. The antibacterial effect of this plant extract could be attributed to the presence of some bioactive compounds like mangiferin [32] and some phytochemical compounds like the flavonoids and resins (Table 3). These secondary metabolites are widely known to possess antibacterial activities [48, 49, 51 and 52]. In a similar work [31], the phytochemical screening of the aqueous extract of M.I stem bark showed the presence of tannins, saponins alkaloids phenols etc but no flavonoids and resins. It should be noted that geographical location and the period of harvest plays important roles on the phytochemical compound present in a plant [32]. This could be the reason why there are differences in phytochemical compounds obtained from the aqueous extract of the M.I stem bark.

The toxicological investigation indicates that the plant extract is not toxic even at a high dose of 5000mg/kg body weight. It was earlier established that any substance with LD50 estimate greater than 2000 mg/kg body weight by oral route may be considered of low toxicity and safe in humans [53, 54]. None of the experimental animals died during the course of the experiment rather they increased in body weight (Table 5). This may explain why this plant extract is used in Cuba as a nutritional supplement. This extract is proposed as both a nutritional supplement (antioxidant) and to prevent disease progress or increase the patient’s quality of life [9]. The haematological parameters (Table 6) show a positive correlation between the control and the test groups. Though there is no statistical significant difference at ($p < 0.05$) between the control and the test groups it appears that at lower doses of the extract the PCV and the haemoglobin concentration (Hb) values are higher than those of the control and the test groups given higher doses. This could mean that lower doses of the plant extract has better effect on the PCV and Hb of the test animals. The result of the haematological parameters suggests that the plant extract does not have any adverse effect on the bone marrow, kidneys and haemoglobin metabolism. The values of the total white blood cell count of the control and the test groups are within the normal range however it appears like the extract elicited increased production of the white blood cells as the groups with higher doses of the extract have higher values of total white blood cells count. This could mean that the extract helps in the rats’ body defense mechanism hence its use as an antimicrobial. This finding agrees with that of other authors like [55] and [56] who stated that there was significant increase in total WBC in the test animals suggesting that the extract may have immunological properties, by stimulating increased production of white blood cells, thereby boosting the defense system of the animals. The result of the biochemical parameters (Table 6) from the test animals shows that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which are biomarkers of hepatocellular injury are within the reference range and are comparable to the values obtained from the control groups. This could mean that the plant extract is not injurious to the animals’ liver. Also the histological tissue section (figure 1) shows an apparently healthy liver. This finding does not agree with the work of Ogbe et al [55] who found a significant elevation in the activities of AST and ALT in the serum of test animals suggesting that aqueous ethanolic extract of *M. indica* stem bark might induce hepatocellular injury in animals. This could be because of the differences in the extracting solvents used. Ethanol may have extracted some compounds injurious to the liver which water did not extract. Also The non significant difference in the levels of sodium ions, potassium ions chloride ions etc (Table) between the test and control groups suggests that this extract may not have interfered significantly with the metabolism of these biochemical parameters sused as markers for kidney function within the duration of the study. The histological tissue section of the kidneys (figure 2) shows normal appearing kidney tissue for both the test and control animals. In conclusion, the crude aqueous extract of MI stem bark has antibacterial activity and appears to have no toxicological side effect on the animals used even at a high dose of 5000mg/kg.

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