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The Histopathological Effect Of Leaf, Stem And Root Bark Extracts Of *morinda mucida* On Some Visceral Organs And Muscles Of Wistar Mice

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The leaf, stem and root bark of *Morinda lucida (Ezeogwu),* are bitter and astringent used in Nigeria in the treatment of fever, malaria, yellow fever, jaundice and dysentery. They are also used as dyestuff. The aim of the study was to investigate and compare histological effects of the leaves, stem and root bark extracts of *Morinda lucida* on some visceral organs and muscles of albino Wistar mice. Acute intraperitoneal toxicity tests were performed for each of the extracts to determine their LD_{50s} using modified Lorke's method. Sub-chronic toxicity study was then carried out by intraperitoneal administration of different doses of the extracts on daily basis to the different groups of male mice for 21 days. The weights of the mice were taken before, during and after administration of the substance at weekly intervals. The animals were subsequently sacrificed and the liver, kidney, stomach, colon and muscle excised for histology processing and analysis. The acute intraperitoneal toxicity result (LD_{50}) revealed *Morinda lucida* leaf, stem and root bark extracts to be lethal at 1,732.1; 1,058.3 and 970.8mg/kg body weight respectively. Microscopic examinations of the kidney, liver, stomach, colon and cardiac muscles showed that the effects of sub-chronic administration of *Morinda lucida* on the liver varied with the type of extracts and was dose dependent. The root extract had higher toxic effect. It had no adverse effect on the kidney, muscles, stomach and colon. This result may form the basis for further trials. It shows that *Morinda lucida extracts* are nontoxic at the dosage and oral route used by local traditional healers for its administration. However, caution is necessary in case of over dose.

Keywords: Morinda Lucida, Histopathological effect, Kepatoprotecitve.

INTRODUCTION

The use of medicinal plants has always been part of human culture and is wide spread in Africa. In some countries, like Ghana, government encourages the use of indigenous forms of medicine, rather than expensive imported drugs. In Nigeria, a huge proportion of the populace depends on herbal medicine because the commercially available orthodox medicines are becoming increasingly expensive and out of reach. Herbs and spices are generally considered safe and have proved to be effective against certain ailments (O'Hara *et al*, 1998). Amongst the medicinal plants commonly used in Nigeria for management or treatment of various types of ailments is *Morinda lucida* Benth.

Morinda lucida is a medium sized tree with a crooked hole and rather short twisted branches. It belongs to the family *Rubiacae.* The bark is rough, grey in colour, flaking off in irregular patches. Its leaves are about 7-15cm long (Burkill, 1997). It is a tropical West African rain forest tree also called Brimstone tree. In Cote d' voire, it is locally known as *Sangogo* or B*ondoutou alongua,* in Ghana it is known as *Twi, Kroma* or *Ewe amake* or *atak ake*, while among the Yoruba natives (South-West Nigeria), it is called *Oruwo* (Daziel, 1937). Among Igbos of South-East Nigeria, it is known as *Eze Ogwu, Akpakwulu Ikenga or Njisi.*

Different parts of the plant have been attributed with diverse therapeutic benefits; for example in Southern Cameroun, cold decoction of the plant leaves is used for treatment of fever. However, in most parts of West Africa, the bitter water decoction of the plant bark, root and leaf are used as bitter tonic and as astringent for dysentery, abdominal colic and intestinal worm infestation. Europeans sometimes use the decoction of the plant root or stem to make "bitters" (Daziel, 1937). It is recommended in the treatment of hypertension and celebral complications showing distinct and tranquilizing effect (Debray et al. 1974). In

some parts of Nigeria, it's stem bark, root and leaves are astringent used in the treatment of fever, malaria, jaundice and ulcerating abscesses, leprosy and gonorrhea (Durodola, 1974). The leaves have also been reported to posses strong trypanocidal and aortic vasorelaxant activities (Asuzu, *et al*; 1990). Further studies have shown that the leaf and stem bark posses anti cancer (Sowemimo, *et al;* 2007), cytotoxic, genotoxic and hepatoprotective (Oduola, *et al*; 2010), antispermatogenic (Raji *et al;* 2005), hypoglycemia and antidiabetic (Daziel, 1973), effects.

Diabetes Mellitus (DM) is a chronic disorder of carbohydrate, lipid and protein metabolism characterized by persistent elevations of fasting blood glucose above 200mg/dl, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action (Murray and Pizzorno, 1997). DM is associated with increased risk of heart disease, stroke, kidney disease, retinopathy, neuropathy, ulceration and gangrene of extremities (Rotshteyn and Zito, 2004). Thus,DM and its associated complications have significant impact on health, quality of life and life expectancy of its sufferers. Recent statistics showed that the global epidemic of diabetes mellitus is worse or greater in developing than the developed countries (Oputa, 2002). For example, India is rated the leading country affected by DM epidemic with an estimate of 19 million diabetic subjects while China and United States of America are rated second and third, respectively (King*et al;* 1998). However, approximately 120 million people are globally affected and this figure is estimated to double by the year 2025 (King*et al;* 1998)

In response to this global challenge, the WHO Expert Committee on diabetes mellitus recommended further evaluation of the folkloric methods of managing the disease because of high morbidity and mortality arising from its attendant complications and draw-backs associated with the use of conventional antidiabetic drugs (Adeneye *et al.,* 2006a). In pursuit of this goal, several medicinal plants are being investigated for their hypoglycemic efficacies. Of the several indigenous plants used in the local treatment of DM among Yorubas (South-West Nigeria) is *Morinda lucida* Benth.

Apart from its medicinal values, alternative cheaper and bio-friendly natural dye had previously been extracted from *Morinda lucida* for the staining of collagen fibres and muscle fibres (Avwioro, 2005). The staining component was described as an anthroquinone, which stains best at an acid pH. Other uses include production of inks, tattoos, and mordant (Burkill, 1997).

Plants produce a diverse range of bioactive molecules making them rich source of different types of medicine (Nair et al; 2005). In recent times, attention has been reverted back to plants as sources of therapeutic agents because of their affordability, availability and relative lower incidence of adverse reaction, compared to modern conventional pharmaceuticals (Karachi, 2006). Therefore there is a need to continuously and scientifically evaluate and document the ethnomedical uses and efficacy of the plant resources of Nigeria. This will be required to identify and develop candidate drugs and to justify or otherwise, the continued administration of such plant extracts to human patients by local traditional doctors (Nwobodo, 2002).

Aims and Objectives

As euphoria about the usefulness of medicinal plants grows, researchers have become aware of the toxicity of their extracts (Sofowara, 1993). While *Morinda lucida* has such diverse traditional medicinal value in Nigeria and other African Countries, there appears to be limited toxicity report on it, and its effect on visceral organs and muscles has not been reported (Raji *et al;* 2005). Since it is possible for *Morinda lucida* to find its way into the human ecosystem as therapeutic agent or dye stuffs, it is possible that bio-accumulation of the substance to a toxic level in living organism can constitute health hazard. Dyes and pigments find their way into human body through materials such as clothing, hair dye and also by ingestion (Okafor and Akpuaka, 2003). Hence this work is undertaken:

1. To determine the intraperitoneal (i.p.) median lethal dose (LD_{50}) of the different

extracts.

2. To evaluate the pathological effects of administering the leaves, stem and root bark extracts of *Morinda lucida* on some visceral organs and muscles of Wistar mice.
3. To compare and contrast the pathological

Fig.1. Photograph of *Morinda lucida* Plant in Anatomy Department, University of Nigeria Enugu Campus compound.

MATERIAL AND METHOD

Plant Materials And Extraction Procedure

The leaf, stem bark and root bark of *Morinda lucida* were obtained from a tree in Anatomy Department, College of Medicine, Enugu Campus. The specimens were authenticated at the *herbarium* of the Department of Botany of University of Nigeria, Nsukka, by the Chief Taxonomist. A voucher specimen was deposited at the *herbarium* for future references. The plant materials were washed with tap water and cut into tiny bits, dried at room temperature under shade for 30 days and hammer milled. The ground samples of leaf, stem and root barks, each weighing 450gm were separately immersed in 1 litre of water fo 72 hours on a mixer to ensure maximum extraction. The extracts were filtered with Whatman number 1 filter paper and the decoctions concentrated to dryness in water-bath maintained at between 60^0C – 70^0C. The extracts obtained were placed in sterile labeled reagent bottles at a temperature of 4^0C until needed for the experiment. From this stock, fresh solutions of the extracts dissolved in distilled water were prepared for administration to the animals.

Experimental Animals

Seventy-eight inbred male adult Wistar mice; weighing 17 – 35gm were obtained from the animal House of University of Nigeria, Nsukka. They were kept in mice cages in a well ventilated house in the Animal House of Anatomy Department, University of Nigeria, Enugu Campus under ambient temperature (25^0C ± 3^0C), 12 hours natural light and 12 hours dark cycle. They were fed *ad libitum* with Guinea feed mixed with Vita Feed and tap water. They were allowed 2 weeks to acclimatize to the environment prior to the commencement of the studies.

Acute Toxicity Test (determination Of LD_{50})

The intraperitoneal acute toxicity study of Morinda lucida aqueous leaf, stem and root bark extracts were carried out in mice using modified Lorke (1983) method for LD_{50} determination. The mice were starved for 24 hours but allowed access to water prior to the commencement of the administration of extracts. The study was diphasic. In phase 1, 6 mice were randomized into 3 groups of 2 mice each and were given 10, 100 and 1,000 mg/kg. b. wt. of the extract. This test was replicated for all cases in order to determine the dose range of the 3 extracts. The mice were observed for signs of toxicity such as paw licking, salivation, stretching of entire body, weakness, sleep, respiratory distress, coma or death in the first 4 hours and subsequently daily for 7 days. The second phase of the study was based on the result of the first phase. Another fresh set of 72 white albino mice were grouped in cages into 18 groups of 4 mice each. The first 6 groups received 100, 250, 500, 1,000, 1,500 and 2,000

mg/kg of the aqueous leaf extracts respectively; the second 6 groups received 10, 100, 500, 800, 1,000 and 1,400mg/kg of aqueous stem bark extract respectively, while the third 6 groups received 10, 100, 500, 800, 1,000 and 1,200 mg/kg of aqueous root bark extract respectively. `These were also observed for signs of toxicity and mortality for the first critical 4 hours and thereafter daily for 7 days. The LD_{50} was calculated using the formula:

$$LD_{50} = \sqrt{(a \times b)}$$

Where a = minimum dose that caused 100% (LD_{100}) death

b = maximum dose that did not cause death; i.e. the geometric mean of the consecutive doses for which 0 and 100 % survival rates were recorded in the second phase.

Sub-chronic Toxicity Study

For the sub-chronic toxicity study, the different animal groups were injected varying doses of *Morinda lucida* extracts (MLE) of the leaf, stem and root bark for 21 days as follows:

Leaf Extracts:

12 mice were divided into three groups A, B and C in 3 cages of 4 mice per cage. They received 100, 300 and 500mg/kg body weight (b.w.) of the substance, respectively by intraperitoneal (i.p.) route.

Stem Bark Extracts:

12 mice were divided into another three groups D,E and F in 3 cages of 4 mice per cage. They received 100, 300 and 500 mg/kg of the substance respectively by intraperitoneal route.

Root Bark Extracts

12 mice were also divided into three groups G,H and I in 3 cages of 4 mice per cage. They received 100, 300 and 500 mg/kg of the substance respectively by intraperitoneal route. Since the tests were done simultaneously, group J served as a general control and received only feed and water *ad libitum* for as long as the experiment lasted.

Weights of the animals were taken and recorded just prior to commencement of administration of the extracts to the animals and at a regular weekly interval throughout the duration of the experiment. The animals were also monitored daily for signs of toxicity. On the 22nd day, 2 animals from each cage were randomly selected and painlessly sacrificed by exposure to inhalation of chloroform anesthetic vapour. Some visceral organs, namely kidney, liver, colon, cardiac muscles and stomach were dissected out from each animal. They were examined grossly, to detect gross anatomical changes arising from treatment with the various concentrations of *Morinda lucida* extracts, before being processed histologically for microscopic examination and analysis.

3.5 Histological Processing

After sacrificing the animals, representative samples of each organ to be studied were carefully dissected out with a standard dissection set. They were appropriately labeled and fixed in 10 per cent netral formol saline for 24hrs and processed by paraffin wax embedding method by dehydrating in series of graded alcohols (70%, 90%, 95% and three changes of absolute ethanol) for 1 hour, 30 minutes in each bath. Clearing was achieved by two changes of chloroform (an ante medium), for 2 hours in each. Impregnation and embedding of tissues were done using molten paraffin wax at 70^0 C. Sections of tissues, 5µm thick were cut using rotary microtome (Sakura, fine tech, Netherlands). Cut sections were attached to slides label using diamond pencil. They were dried (at 65^0 C for 45 minutes on a hot plate), and stained by Haematoxylin and Eosin (H and E) method. Periodic Acid Schiff (P.A.S) method was also used to demonstrate intestinal mucin. They were thereafter examined in light microscope and photomicrographed subsequently.

Statistical Analysis

Data were expressed as mean±SEM. Statistical significance between the various groups was determined using student t-test and one way ANOVA. The significance of the difference between the mean of the control and the administered groups was considered as $p<0.05$.

RESULTS

Acute Toxicity Test:
Clinical observation and behavioural changes
Leaf Extracts

Table 1: Determination Of LD_{50} Of Leaf Extract

Cages	No of animals	Dosage mg/kg (b.w.)	No Dead	No Alive
A	4	300	0	4
B	4	500	0	4
C	4	700	0	4
D	4	1000	0	4
E	4	1,500	0	4
F	4	2,000	4	0

$Ld_{50} = \sqrt{(a \times b)} = \sqrt{(1{,}500 \times 2{,}000)}$ 1,732.1mg.kg

At a dose of 2000mg/kg the animals were weak and sluggish. There was evidence of diarrhoea and 100% death within 24hrs after the administration of the substance. The LD_{50} was 1,732.1mg/kg.

Stem Bark Extracts
Table 2: Determination Of LD_{50} of Stem Bark Extract

Cages	No of animals	Dosage mg/kg (b.w.)	No Dead	No Alive
A	4	10	0	4
B	4	100	0	4
C	4	500	0	4
D	4	800	0	4
E	4	1,000	3	1
F	4	1,400	4	0

$Ld_{50} = \sqrt{(a \times b)} = \sqrt{(1{,}400 \times 800)} = 1058.3$mg/kg

At a dose of 1400mg/kg, anal bleeding, abdominal enlargement, ruffled hair and 100% death occurred within 24hours after administration of the substance. The LD50 was 1058.3mg/kg.

Root Bark Extracts
Table 3: Determination Of LD_{50} of Root Bark Extract

Cages	No of animals	Dosage mg/kg (b.w.)	No Dead	No Alive
A	4	10	0	4
B	4	100	0	4
C	4	500	0	4
D	4	800	0	4
E	4	1,000	2	2
F	4	1,200	4	0

$Ld_{50} = \sqrt{(a \times b)} = \sqrt{(1{,}200 \times 800)} = 970.8$mg/kg

At a dose of 1,200mg/kg there was evidence of sluggishness, diarrhea and death within 24hrs after administration of the substance. The LD_{50} was 970.8mg/kg.

Table 4: Sub-Chronic Studies STEMLeaf Extracts

S/N	Group	Dosage mg/kg	Behavioural changes	Histological Findings	
				Organs	Remarks
1	A	100	NAD	Colon	No Abnormality Dictated (NAD)
				Liver	NAD
				Stomach	NAD
				Muscle	NAD
2	B	300	NAD	Colon	NAD
				Kidney	NAD
				Liver	NAD
				Stomach	NAD
				Muscle	NAD
					NAD
					NAD
3	C	500	NAD	Muscle	NAD
				Colon	NAD
				Kidney	NAD
				Stomach	NAD
				Liver	Mild to moderate Periportal Lymphocytic Infiltration.

There were no obvious behavioural changes among both treatment and control animals). Animals gained in weights until sacrificed. The gross specimens also appeared normal at necropsy.

Table 5: Sub-Chronic Studies of Bark Extracts

S/N	Group	Dosage mg/kg	Behavioural changes	Histological Findings Organs	Remarks
1	D	100	NAD	Colon	NAD
				Kidney	NAD
				Stomach	NAD
				Muscle	NAD
				Liver	NAD
2	E	300	NAD	Colon	NAD
				Kidney	NAD
				Stomach	NAD
				Muscle	NAD
				Liver	NAD
3	F	500	NAD	Colon	NAD
				Kidney	NAD
				Stomach	NAD
				Muscle	NAD
				Liver	Severe Lymphocytic Infiltration of the portal area

No behavioural changes were observed. At a dose of 100 and 300mg/kg there was significant weight gain. At 500mg/kg, weight loss was significant. However, the gross anatomy specimens of liver, kidney, stomach, colon and muscles appeared normal at necropsy.

Table 6: Sub - Chronic Studies Root Bark Extracts

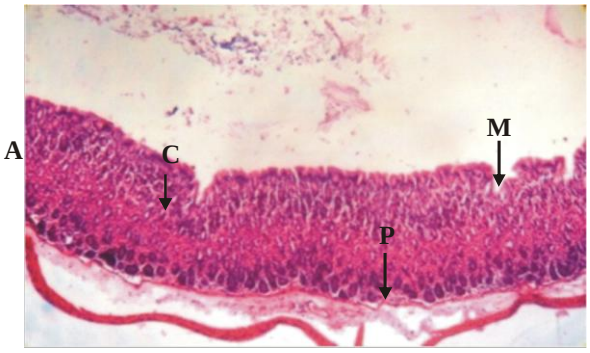
S/N	Group	Dosage mg/kg	Behavioural changes	Histological Findings Organs	Remarks
1	G	100	NAD	Colon	NAD
				Stomach	NAD
				Kidney	NAD
				Muscle	NAD
				Liver	NAD
2	H	300	NAD	Colon	NAD
				Kidney	NA D
				Stomach	NAD
				Muscle	NAD
				Liver	NAD
3	I	500	NAD	Colon	NAD
				Kidney	NAD
				Stomach	NAD
				Muscle	NAD
				Liver	Severe Lymphocytic Infiltration of the portal tract.

At a dose of 500mg/kg the animals were generally weak with ruffled hairs. At doses of 100 and 500mg/kg there were evidence of weight losses at the time of sacrifice. No gross anatomical changes were evident at necropsy.

Histological Findings

Leaf Extracts:

At 500mg/kg the liver exhibited a mild periportal lymphocytic infiltration. The kidney, stomach, muscles and colon appeared as normal as control in the H&E stained sections. The PAS technique on the colon showed normal morphological pattern of goblet cells.



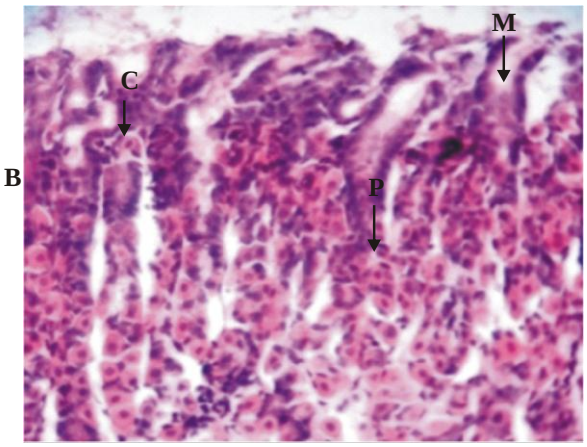
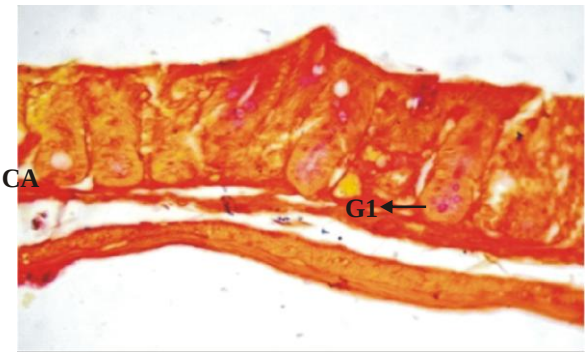


Fig 2 (A and B) light micrographs of stomach of mice.

(A). **Control**: normal of stomach devoid of visible lesions. Normal gastric glands namely (M) mucus neck cells, (P) parietal cells (C) peptic cells.

(B) Stomach of mice administered 500 mg/kg b.wt. of substance for 21 days. There is no abnormality detected.



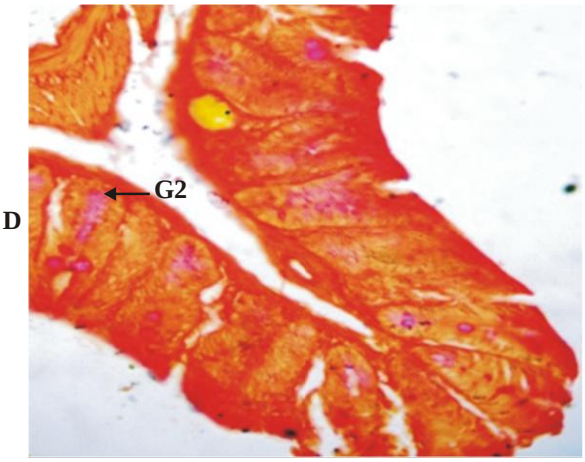
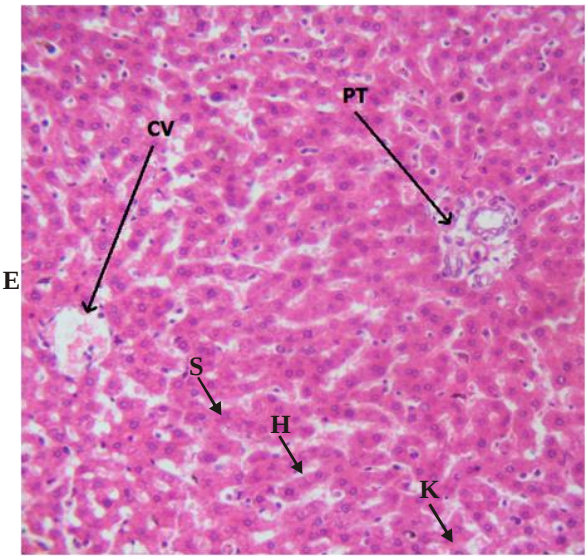


Fig 2 (C and D) light micrograph of colon of mice demonstrating goblet cells stained by PAS method.

C: Control. Normal architecture. Goblet cells (G1) appears magenta

D: Colon of mice administered 500mg/kg. b.wt. of substance for 21 days. There are no histopathological changes observed.



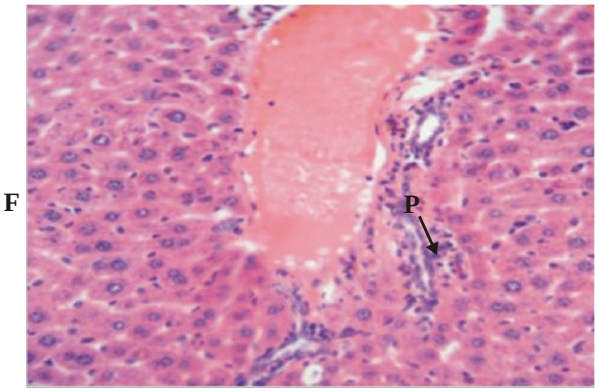


Fig 2 (E and F). Light micrographs of liver of mice.
(E) Control: liver of mice showing normal architecture of hepatocyte (H), hepatic sinusoids (S), Kupfer cells (K) and portal area (P).
(F) Liver of mice administered 500mg/kg. b.wt. of leaf extract for 21days. There is mild periportal lymphocytic infiltration (arrows-P).

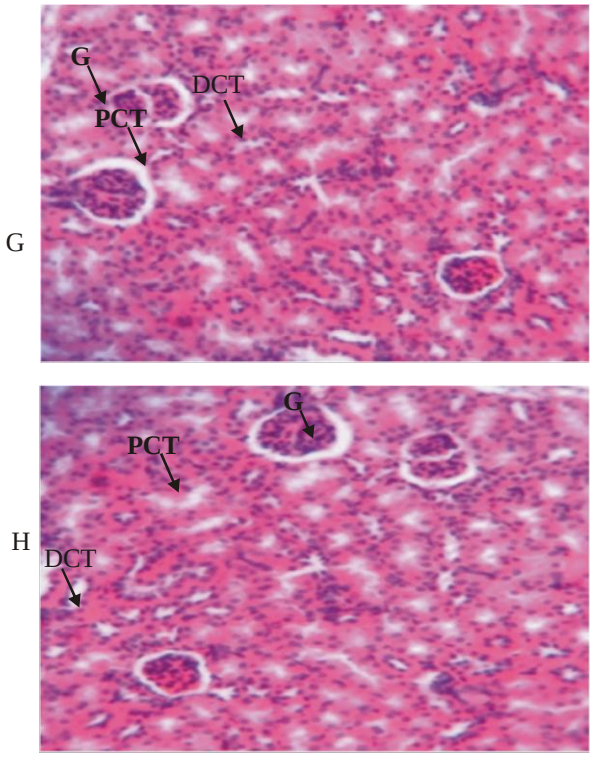


Fig 2 (G and H). Light micrographs of kidney of mice.
(G). Control section showing normal glomerulus (G), normal proximal convoluted tubule (PCT) and normal distal convoluted tubule (DCT).
(H). Kidney section of mice administered 500mg/kg. b.wt. of leaf extract for 21 days showing normal histological architecture.

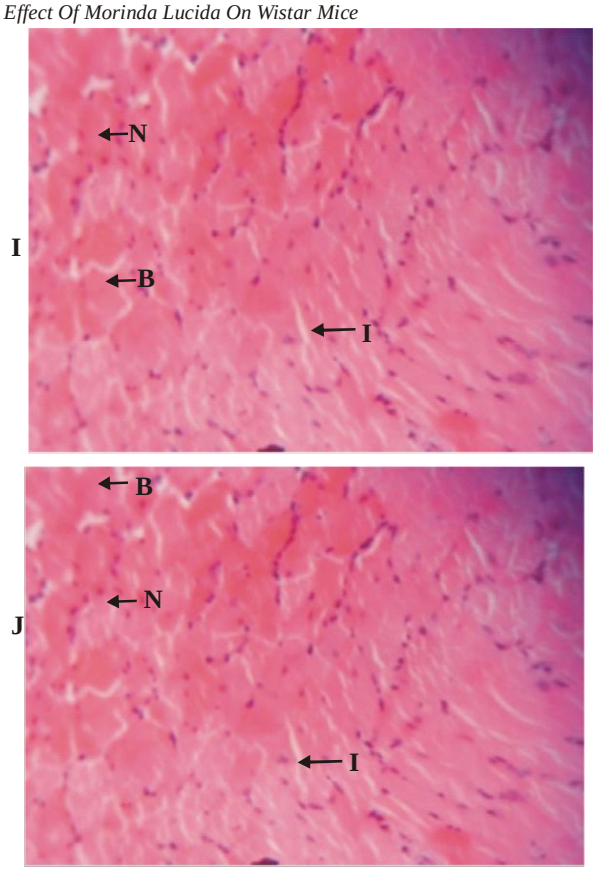
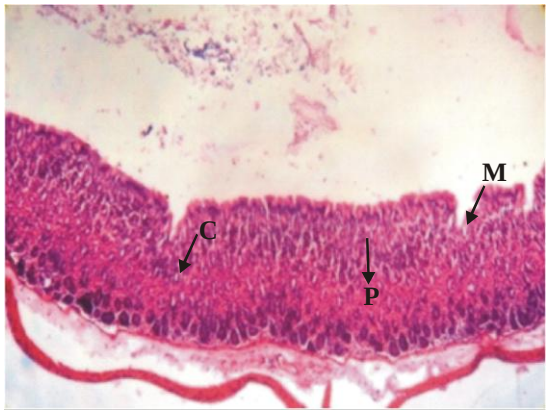


Fig 2. (I and J). Light micrographs of cardiac muscles of mice.
(I). Control: showing normal nuclei (N) and intercalated disc (I). Branching fibers and three dimensional arrangement of the fibers is evident.
(J). Muscle of mice administered 500mg/kg. of leaf extract. There is no abnormality detected.

Stem Bark Extracts:

At 500mg/kg the liver showed severe lympocytic infiltration of the portal area, while the stomach, colon, kidney and muscles remained normal in the H&E stained sections. PAS reaction on colon showed a normal morphological pattern of goblet cells.



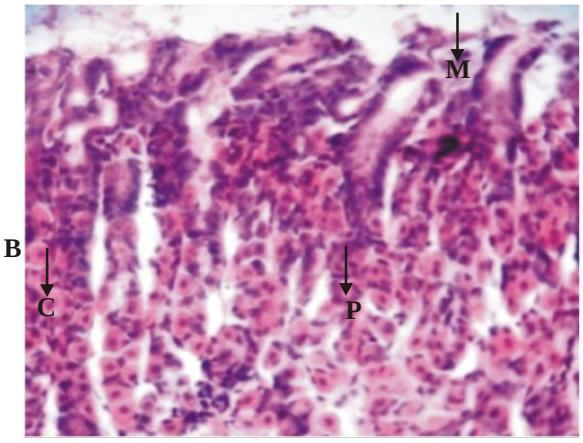


Fig 3. (A and B): light micrographs of stomach of mice.
(A). Control: normal stomach devoid of visible lesions. Normal gastric glands - (M) mucus neck cells, (P) parietal cells (C) peptic cells.
(B) Stomach of mice administered 500 mg/kg b.wt. of substance for 21 days. There is no abnormality detected.

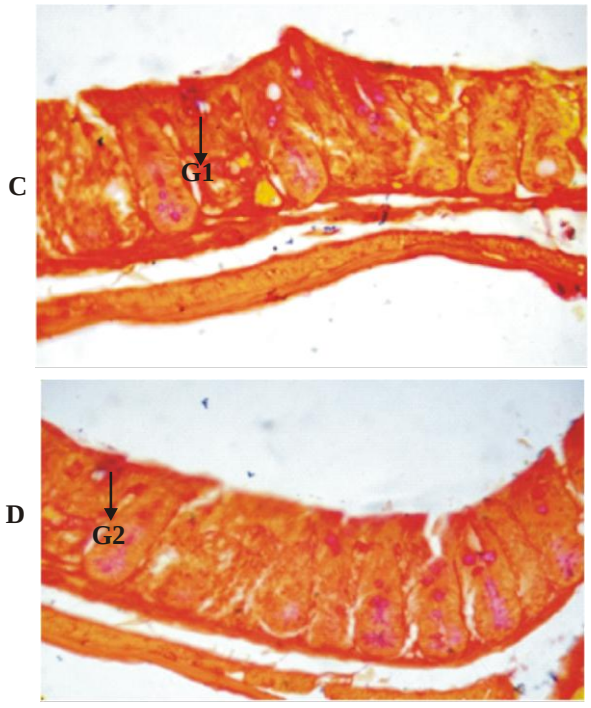
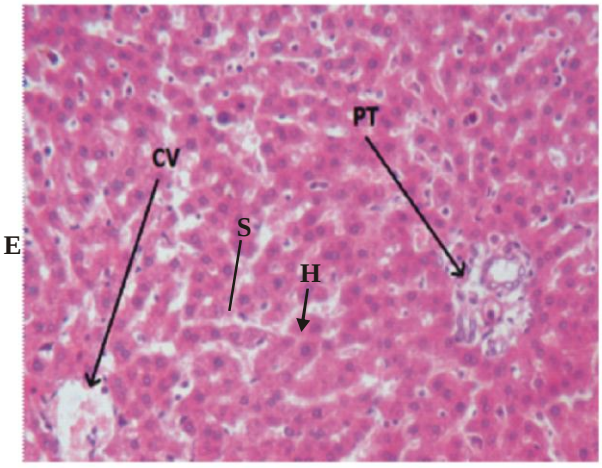


Fig 3. (C and D) light micrograph of colon of mice demonstrating goblet cells stained by PAS method.
(C) Control: Normal architecture. Goblet cells (G1) appears magenta
(D) Colon of mice administered 500mg/kg. b.wt. of substance for 21 days. There are no histopathological changes observed.



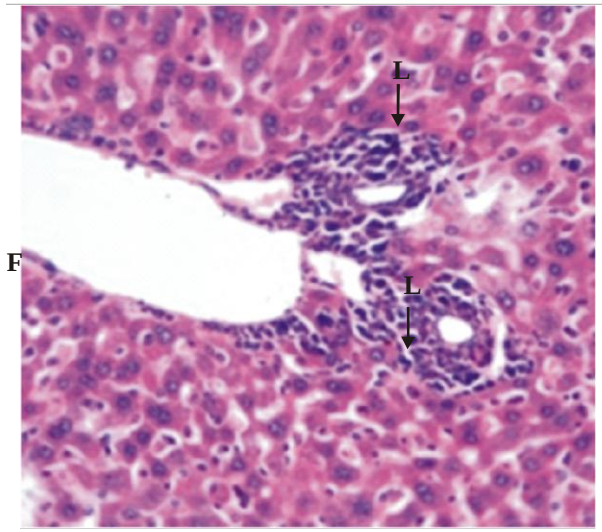
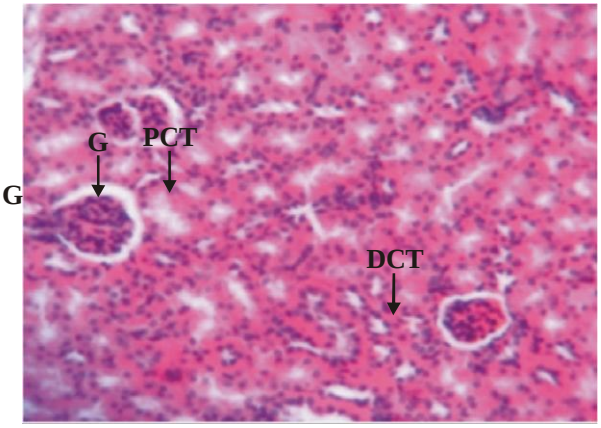


Fig 3. (E and F). Light micrographs of liver of mice.

(E) Control: liver of mice showing normal architecture of hepatocyte (H), hepatic sinusoids (S), and portal area (PT).

(F) Liver of mice administered 500mg/kg. b.wt. of stem bark extract for 21days. There is severe lymphocytic infiltration of portal area (arrow L)



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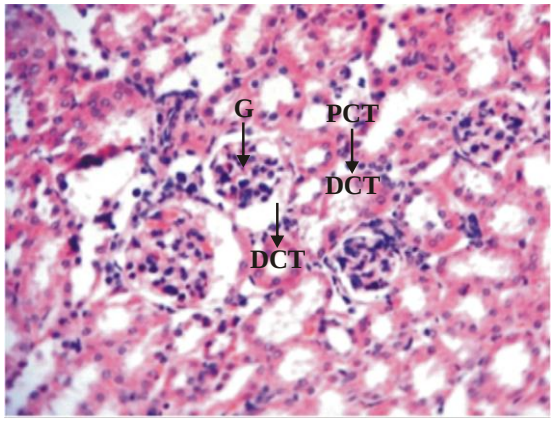
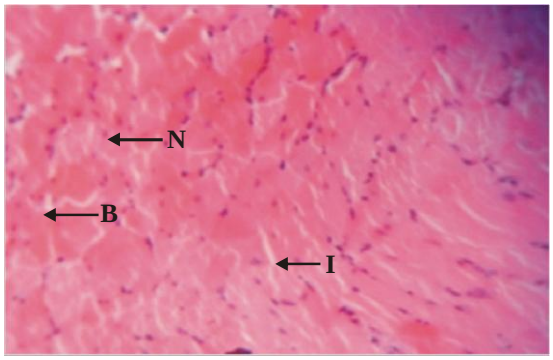


Fig 3. (G and H). Light micrographs of kidney of mice.
(G). Control section showing normal glomerulus (G), normal proximal convoluted tubule (PCT) and normal distal convoluted tubule (DCT).
(H). Kidney section of mice administered 500mg/kg. b.wt. of stem bark extract for 21 days. No abnormality detected.

I



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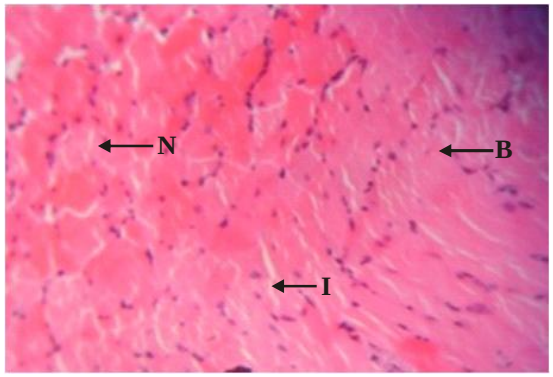
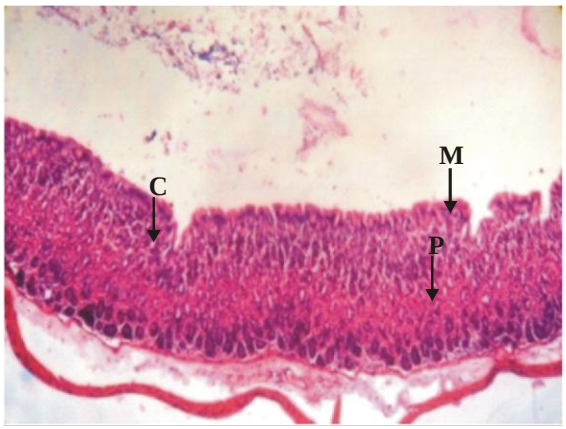


Fig 3. (I and J). Light micrographs of cardiac muscles of mice.
(I). Control: showing normal nuclei (N) and intercalated disc (I). Branching fibers and three dimensional arrangement of the fibers is evident.
(J). Muscle of mice administered 500mg/kg. of stem bark extract. There is no abnormality detected.

ROOT BARK EXTRACT

At 500mg/kg, there was severe lymphocytic infiltration of the hepatic portal tract. The stomach, colon, kidney and smooth muscles were normal in H & E stained sections. The PAS technique on colon showed normal morphological pattern of goblet cells.

A



B

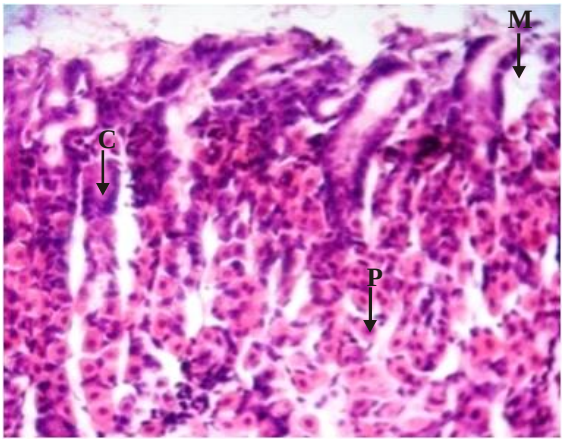
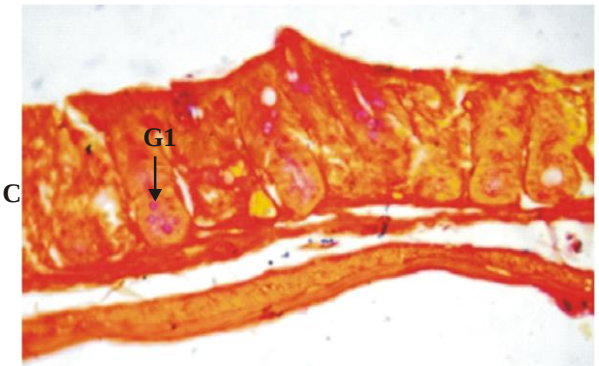


Fig 4. (A and B): light micrographs of stomach of mice.
(A). Control: normal stomach devoid of visible lesions. Normal gastric glands - (M) mucus neck cells, (P) parietal cells (C) peptic cells.
(B) Stomach of mice administered 500 mg/kg b.wt. of substance for 21 days. There is no abnormality detected.

C



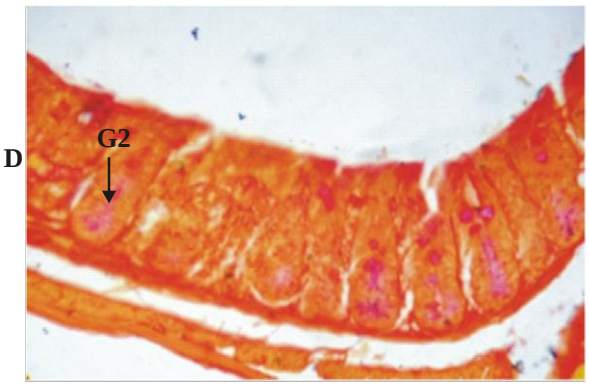


Fig 4. (C and D) light micrograph of colon of mice demonstrating goblet cells stained by PAS method.
(C) Control: Normal architecture. Goblet cells (G1) appears magenta
(D) Colon of mice administered 500mg/kg. b.wt. of substance for 21 days. There are no histopathological changes observed.

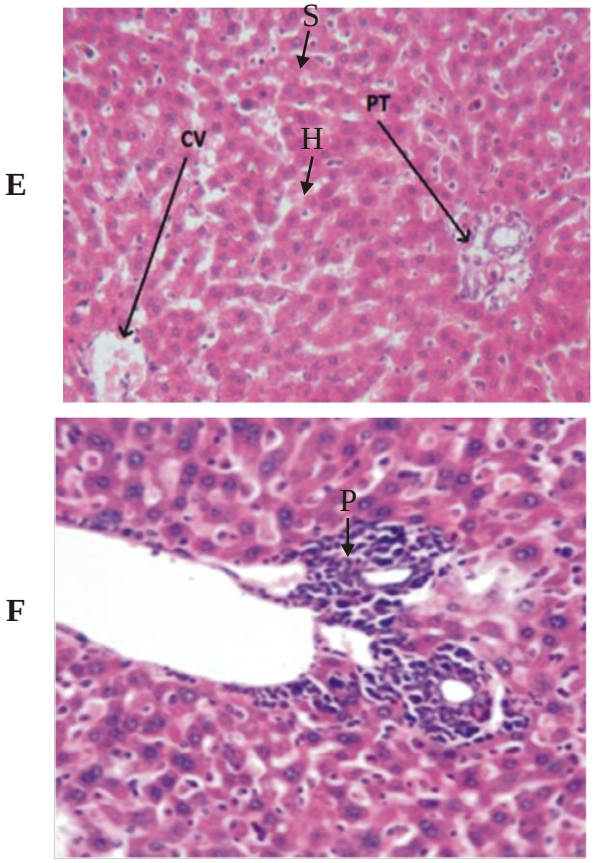


Fig 4. (E and F). Light micrographs of liver of mice.
(E) Control: liver of mice showing normal architecture of hepatocyte (H), hepatic sinusoids (S), and portal area (PT).
(F) Liver of mice administered 500mg/kg. b.wt. of root extract for 21days. There is severe lymphocytic infiltration of portal area (arrow P)

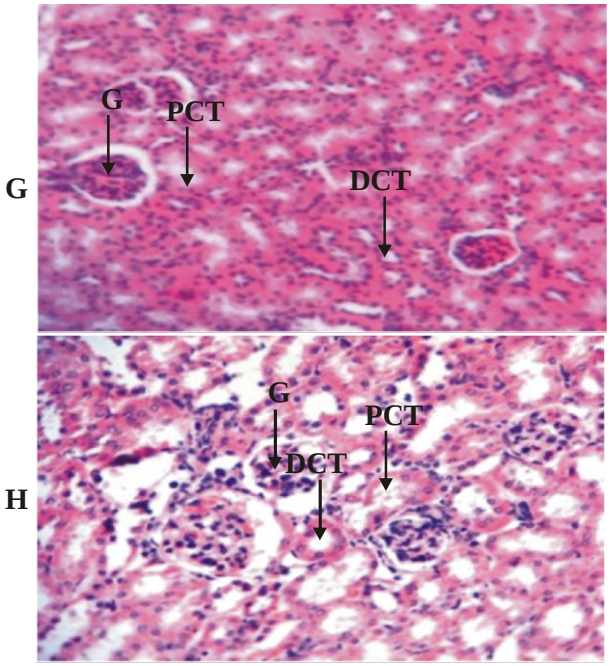


Fig 4. (G and H). Light micrographs of kidney of mice.
(G). Control section showing normal glomerulus (G), normal proximal convoluted tubule (PCT) and normal distal convoluted tubule (DCT).
(H). Kidney section of mice administered 500mg/kg. b.wt. of root extract for 21 days. No abnormality detected.

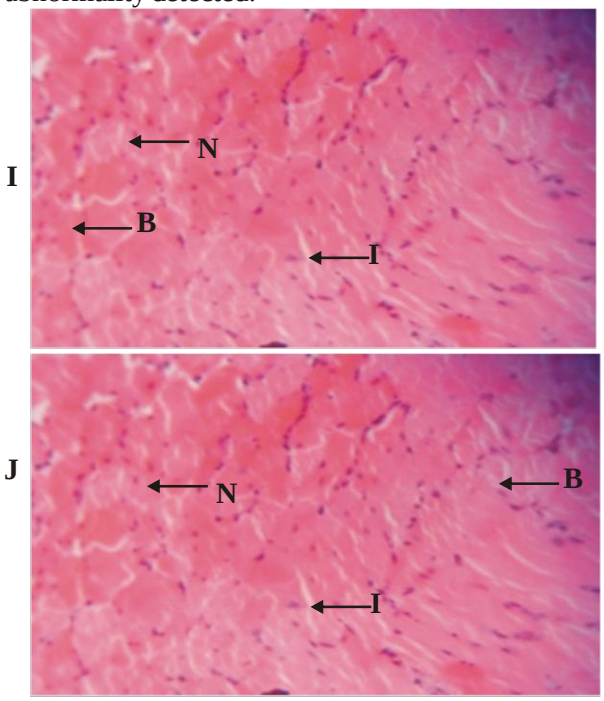


Fig 4. (I and J). Light micrographs of cardiac muscles of mice.
(I). Control: showing normal nuclei (N) and intercalated disc (I). Branching fibers and three dimensional arrangement of the fibers is evident.
(J). Muscle of mice administered 500mg/kg. of root extract. There is no abnormality detected.

Table 7
MEAN ±SD WEIGHT OF THE TEST AND CONTROL GROUPS BEFORE AND AFTER LEAF EXTRACT ADMINISTRATION

	100mg/kg	300mg/kg	500mg/kg	CONTROL
Week 0 (kg)	31.70±1.49	38.07±4.57	27.95±0.50	28.38±1.64
Week 1 (Kg)	31.07±1.37	38.83±5.23$^{\alpha}$	28.00±0.28$^{\beta}$	28.83±1.57
Week 2 (kg)	31.60±1.35	38.37±4.51	28.95±0.15$^{\beta}$	32.63±2.47
Week 3 (kg)	32.17±1.04	39.87±5.26	28.60±1.40$^{\alpha\beta}$	31.13±1.53
ANOVA (Week0, 1, 2 and 3)	P>0.05	P>0.05	P>0.05	

* COMPARED WITH BASE LINE MEAN VALUE
α COMPARED WITH CONTROL
β ONE-WAY ANOVA (FOR 100mg, 300 and 500mg)
ONE-WAY ANOVA (FOR Baseline, 2nd, 3rd, and 4th weighing)

All the test groups showed no statistical significant differences (p>0.05) in mean weight values at 1,2 and 3 weeks after extract administration when compared with the baseline mean weight values (week 0). However, when compared with control group, only the group given 300mg/kg leaf extract showed statistical significant increase (P<0.05) at the first week. Also, there were no statistical significant time-dependent change in the mean weights of animals placed on 100, 300 and 500mg/kg body weight of leaf extract from onset of the experiment to the end or third week of extract administration, while there were statistical significant decrease (p<0.05) in mean weight with increasing concentration of the extract at week 1, 2 and 3 respectively.

Table 8
MEAN ±SD WEIGHT OF THE TEST AND CONTROL GROUPS BEFORE AND AFTER STEM BARK EXTRACT ADMINISTRATION

	100mg/kg	300mg/kg	500mg/kg	CONTROL
Week 0 (kg)	27.67±0.15	29.23±2.03	30.00±0.71	28.38±1.64
Week 1 (Kg)	26.97±1.97	25.97±2.15	29.65±3.89$^{\beta\beta}$	28.83±1.57
Week 2 (kg)	27.50±1.32	30.13±3.37	29.30±4.95$^{\beta\beta}$	32.63±2.47
Week 3 (kg)	29.53±1.57	29.90±1.15	28.90±4.10$^{\beta\beta}$	31.13±1.53
ANOVA (Week 0-3)	P>0.05	P>0.05	P>0.05	

Comparison of mean baseline weights at week 0 with that of Week 1, 2 and 3 after stem bark extract administration showed no statistical significant difference (p<0.05) in the entire test groups. One-Way ANOVA showed no statistical significant difference (p>0.05) when the various periods of exposure were compared while with increase in concentration, there were statistical significant increase in mean weight of the test groups at week 1(p<0.01), Week 2 (p<0.01) and decrease at Week 3 (p<0.05) respectively of the stem bark extracts administration.

Table 9
MEAN ±SD WEIGHT OF THE TEST AND CONTROL GROUPS BEFORE AND AFTER **ROOT BARK EXTRACT ADMINISTRATION**

	100mg/kg	300mg/kg	500mg/kg	CONTROL
Week 0 (kg)	27.10±0.53	17.20±0.36	19.25±0.35	18.38±3.28
Week 1 (Kg)	26.10±0.87	17.50±0.26	$_{\beta\beta\beta}$21.75±0.21*$^{\alpha}$	18.83±3.13
Week 2 (kg)	27.80±0.53	17.17±0.21	22.50±0.71$^{\beta\beta\beta}$	22.63±4.06
Week 3 (kg)	18.97±0.83*	19.33±0.42**	16.65±0.92$^{\beta\beta}$	21.13±2.71
ANOVA (Week 0-3)	$^{\#\#}$P>0.05	$^{\#\#\#}$P>0.05	$^{\#\#}$P>0.05	

Only the groups given 100mg/kg and 500mg/kg recorded statistically significant decrease ($p<0.01$) and increase ($p<0.05$) respectively in mean weight at Week 1 when compared with the control group. On comparison of the baseline mean weight values with the weight at 1,2 and 3 weeks of root extract administration, the animals on 100mg/kg and 300mg/kg recorded statistically significant reduction ($p<0.05$) and rise ($p<0.01$) respectively at Week 3 while those on 500mg/kg showed a statistical significant increase ($p<0.05$) at week 1 of administration. No statistically significant time dependent change in weight was observed in any of the test groups but a statistical significant decrease in weight was observed at week 1($p<0.001$), week 2 ($p<0.001$) and week 3 ($p<0.01$) with increased dosage.

DISCUSSION

The results of acute intraperitoneal toxicity (LD_{50}) of the various plant extracts were obtained as 1,732.1mg/kg, 1058.3mg/kg and 970.8mg/kg for the leaf, stem and root bark extracts respectively. However, Odutola *et al* (2010) recorded that the oral LD_{50} for the leaf extract was higher than 6,400mg/kg in mice; and Adeneye and Agbaje in an acute oral toxicity study, documented the leaf extract to be non-lethal in rats at 2000mg/kg. Our finding was corroborated by the intraperitoneal LD_{50} value of 2000mg/kg obtained for the 50% methanol extract of the dried leaves of MLE obtained by Asuzu and Chineme (1990) in their earlier study.

According to Bruce (1985, 1987) and the American Society for testing and materials (1987), any chemical substance with LD_{50} estimate greater than 2000-5000mg/kg oral route could be considered of low toxicity and safe. For dyestuff, values above 250mg/kg is also acceptable (Zollinger, 1991). Higher value of LD_{50} shows reduced toxicity. Therefore the values of LD_{50s} of the various extracts obtained in this study are within the safe range. The index of acute toxicity is the LD_{50}. However, LD_{50} should not be regarded as a biological constant, since differing results are obtained on replication or when the determinations are carried out in different laboratories due to many variables such as animals' species and strain, age, gender, diet, bedding, ambient temperature and time of day (Lorke 1983). This may explain the difference between the values of the LD_{50} obtained in this work, and that obtained by Asuzu and Chineme for the leaf extracts.

The results of the sub-acute toxicological study for the leaf extracts and stem bark were similar as no obvious behavioral changes were observed among both administered and control groups. The animals rather gained in weights until sacrificed. This suggests that the intake of the substance does not influence changes in body weight, hence, the feeding habit of the administered group was not impaired by the substance. Furthermore, the autopsy findings revealed that the kidney, liver, stomach, colon and muscles were free from

pathological states.

For the root bark extracts, toxic signs of weakness and ruffled hairs were apparent at 500mg/kg. There was evidence of weight loss at the time of sacrifice. Sofowora (1993) recorded that the root extracts of the plant has some cytotoxic effects. Compounds with cytotoxic activities have the potential for adverse effects to the human body. The phytochemical analysis of MLE revealed the presence of tannins and saponins. Tannins are responsible for most behavioral toxicities induced by medicinal plants (Muyibi *et al;* 1999), while saponins promote cellular proliferation (Zimudzi and Cardon, 2005). At a dosage of 500mg/kg all extracts manifested varied degrees of histopathological changes in liver sections. For the leaf extract, there was a mild periportal lymphocytic infiltration; while for the stem and root bark extracts there was severe lymphocytic infiltrations of the portal area.

CONCLUSION

In conclusion, this study demonstrates that the ingestion of *Morinda lucida* extracts by human patients may be safe but they must be taken with caution since they contain some toxic principles such as saponins with potentials of residual deleterious effects on the liver when taken in over dose proportion. Contrary to the views held by Odutola *et al (* 2010), histological findings pointed to diverse toxic effects of the leaf, stem bark and root bark extracts; the root bark and the stem bark being comparatively more toxic.

RECOMMENDATION

Isolation of the active principles would constitute areas of future research (Adeneye and Agbaje, 2008). Further studies aimed at elucidating the activities of *Morinda lucida* extract would be worthwhile in order to determine and establish their potential deleterious effects or otherwise on the liver and other vital organs

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