Reassessment of Acute and Chronic Toxicity Effects of Aqueous Leaf Extract of *Morinda Lucida* in Rattus Norvegicus

S. A. Saganuwan[*,1], A. D. Aondoaver[1] and I. T. Roman[2]

[1]*Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria*

[2]*Department of Animal Health and Production, College of Veterinary Medicine, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria*

Abstract: In view of preponderance of medicinal uses of *Morinda lucida* there is need to reassess its toxicity potential. Phytochemical analysis of *Morinda Lucida* leaf powder was carried out to determine the therapeutic and toxic principles of the plant. Acute and chronic effects of aqueous extract of *Morinda lucida* leaf were studied in *Rattus norvegicus*. The phytochemical analysis revealed the presence of flavonoids, glycosides, saponins, anthraquinones, tannins, and reducing sugar, whereas steroids were absent. Proximate analysis yielded moisture (8.88%), dry matter (91.12%), ash (18.71%), fat and oil (25.16%), nitrogen free extract (34.30%), crude fibre (12.45%) and crude protein (10.50%). Median lethal dose (LD_{50}) was estimated to be >16,265mg/kg body weight. Haematological analysis revealed significantly (P<0.05) increased packed cell volume, erythrocytes, haemoglobin, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. However white blood cells decreased significantly (P<0.05) at dose level of 1,626.5mg/kg. But, alanine aminotrasferase, glucose, creatinine, total protein, bilirubin, sodium ion, bicarbonate ion and chloride ion decreased significantly (P<0.05). Urea increased significantly (P<0.05). Therefore, aqueous extract of *Morinda lucida* is safe at the test doses of 16.26 and 162.65mg/kg body weight when administered for 28 days with kidney degeneration and mild to moderate deleterious effects on lung, liver, heart and spleen at dose level of 1,626.5mg/kg bodyweight of the extract.

Keywords: Deleterious effect, kidney, Rattus norvegicus, Morinda lucida.

INTRODUCTION

Morinda lucida occurs from Senegal to Sudan and Southwards to Angola and Zambia, sometimes planted around villages in Nigeria. It grows in grassland, exposed hillsides, thickets, forest, often on termite mounds, sometimes in areas, which are regularly flooded, from sea level up to 1300m altitude. Evergreen small to medium-sized tree, up to 9-18m or 25m tall in coastal areas of Ivory Coast, with bole and branches often crooked or gnarled or straight, sometime short, 20-30cm in diameter. Bark is smooth to roughly scaly, grey to brown colour often with distinct purple layers. Leaves are opposite, simple and entire [1]. Inflorescence is a staked cup-shaped gland. Flowers are bisexual and the fruits are drupe several arranged together into an almost globuse succulent syncarp [2]. *Morinda lucida* comprises about 80 species and occurs throughout the tropics. In Africa, 5 species are found. The comparatively small flowering and fruiting heads on long slender peduncles are distinct characteristics of *Morinda lucida*. Many species including those from Africa are important medicinal plants, widely applied against various kinds of fevers and infections [3].

*Address correspondence to this author at the Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria;
Tel: +2347039309400; Fax: +2348027444269;
E-mail: pharm_saga2006@yahoo.com

Morinda lucida reduced contractility of uterine smooth muscle in both pregnant and non-pregnant as well as blocking contractile response to oxytocin and acetylcholine in uterine smooth muscle of pregnant and non-pregnant mice [4]. The bitter roots are used in flavoring for food and alcoholic beverages and in Nigeria; they are popularly used as chewing sticks. The leaves contain high levels of mercury, cadmium, arsenium and lead [5]. Decoctions, infusions and plasters of roots, bark and leaves are recognized remedies against different types of fevers, including yellow fever, malaria, trypanosomosis and feverish conditions during parturition [6]. The plant is also used for the treatment of diabetes, hypertension, cerebral congestion, dysentery, stomachache, ulcers, leprosy and gonorrhea. Either bark or leaf decoction is applied against itch and ringworm. The root is yellow hence the name brimstone tree. It is resistant to fungi, termites and other insects. Aqueous extract showed anti-inflammatory, anti-fever and promoted gastric emptying and intestinal motility [7]. Inhibitory effects on tumours in mice have also been reported. A leaf extract of *M. lucida* caused 100% mortality in the fresh water snails, Bulinus globulus at a concentration of 100ppm. It also showed antispermatogenic [8] antibacterial, antidiabetic [9] and antihelmintic activities against Trichostrongylus colubriformis [10]. Ursolic acid and oleanolic acid extracted from methanol extract of *Morinda lucida* exhibited significant *in vitro* and *in vivo* antiplasmodial

activity. In West Africa, the root of *M. Lucida* is sold in local markets and shops, both as dyestuff and medicine. Leaves and twigs are sold in markets as medicine tonic for young Children. The methanolic extract of the plant yielded steroids, terpenoids, cardiac glycosides, alkaloids, saponins, tannins and flavonoids [11]. From the wood and bark of *Morinda lucida,* 18 anthraquinones have been isolated including the red colorants 1-methylalizarin, rubiadin and its derivatives including, sorankidiol, nordamnacanthal, morindin, murijistin and purpuroxanthol. Two anthraquinones, Oruwal and Oruwalol, have also been discovered, these give a yellow colour and possibly are intermediates in the formation of anthraquinones. Tannins, flavonoids and saponosides have been isolated. The plant yielded two triterpenic acid, ursolic acid and oleanolic acid [12], damnacanthal, digitolutein and rubiadim-1-methyl ether [3]. With reference to medicinal uses of Morinda lucida, there is need to reassess its toxicity effects with a view to establishing its safety.

MATERIALS AND METHODS

Experimental Animals

A total of forty-seven (47) rats of either sex which weighed between 35 and 92g were obtained from the animal house of the College of Medical Sciences of Benue State University Makurdi, Nigeria. All the rats were fed a commercial rat feed (Grower ®) produced by Grand Cereals and Oils Limited (GCOML) Jos, Nigeria. Clean water was provided ad libitum. The animals were handled according to international guiding principles for biomedical research involving the use of animals [13] as certified by animal ethic-committee of Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Agriculture Makurdi Nigeria given the permit number, P/No. 201106.

Collection of the Plant Materials

The plant materials (leaves) used for the study were obtained from Howe clan unit of Gwer-East Local Government Area of Benue State, Nigeria. The plant was collected in the first week of October 2010 and identified by a botanist in the Department of Biological Science, Ahmadu Bello University Zaria where a voucher specimen with voucher number (V/No. 1862) has been deposited.

Aqueous Extraction of *Morinda lucida* Leaf

The plant materials (leaves) collected were air dried to a constant weight under an open shade and pulverized with the help of mortar and pestle to fine powder. Fifty (50) grammes of *Morinda lucida* leaf powder was dissolved in 1000ml of distilled water in a jar. The mixture was thoroughly shaken intermittently throughout the period of extraction using a stirrer, then allowed to stand for 72 hrs. The mixture was filtered with a Whatman filter paper No 1 into measuring cylinder and concentrated at 50 ^{o}C in an incubator and stored in a refrigerator until required for use [14].

Preparation of Stock Solution

Ten (10) grammes of the extract of *M. lucida* leaf obtained from aqueous maceration was measured and placed in a beaker and 90ml of distilled water added to prepare 10% stock solution that was stored in a refrigerator at 4^{o}C until required.

Phytochemical Analysis

The aqueous leaf extract of *Morinda lucida* was evaluated for the presence of total glycosides, saponins, tannins, steroids, flavonoids, anthraquinones, and reducing sugars using the methods described by Brain and Tuner [15].

Proximate Analysis

Proximate analysis was carried out on *Morinda lucida* leaf powder to determine the percentage yield of carbohydrate, protein, lipid, nitrogen free extract, ash and moisture using the methods [16, 17]. Kjeltech machine, model Tecator (1002 Distilling Unit) made in Sweden was used to analyze the sample.

Acute Toxicity Study

The up and down procedure was adopted to estimate median lethal dose (LD_{50}) of the plant extract. Fourteen (14) female rats of about 7 weeks old, which weighed 35 $\pm$ 2.5g were used for median lethal dose (LD_{50}) estimation. Five rats were administered 2,000mg/kg body weight by gavage. The animals were initially observed for 48 hours and thereafter for a period of 12 days. Having observed the survival of the five rats, a dose progression of 3.2 factors yielded 3,585mg/kg. Therefore, the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th and 9th rat were sequentially administered oral aqueous leaf extract of *Morinda lucida* at dose levels of

3585, 5170, 6755, 8340, 9925, 11510, 13095, 14680 and 16465mg/kg body weight respectively. All the rats were observed for a period of 48 hours and thereafter for 12 days for toxicity signs including mortality. The observed toxicity signs were recorded and the median lethal dose (LD_{50}) of the extract estimated. The continuous sequential dosing of the animals was necessitated by their survival.

Chronic Toxicity Study

The method of [18] was adopted for selection of doses used for subchronic toxicity study. Thirty-two (32) rats of either sex that weighed 92 $\pm$ 2.5g of 10weeks old used for this study were divided into four groups, eight per group administered by gavage 2ml containing 0, 16.26, 162.65 and 1626.5mg/kg body weight of aqueous leaf extract of Morinda lucida for a period of 28 days. Twenty four hours after the administration of last dose of the extract, one milliliter (1ml) of blood was obtained from the tail vein of each rat with the help of needle and syringe and placed in a tube containing ethylene diamine tetraacetate (EDTA) anticoagulant. The anticoagulant blood was used for determination of haematological parameters. The remaining blood samples were centrifuged to obtain plasma for biochemistry.

Haematological Parameters

The haematological parameters determined include erythrocyte (rbc) count, packed cell volume (pcv), heamoglobin concentration (hbc), Mean corpuscular volume (mcv), mean corpuscular heamoglobin concentration (mch), white blood cells (wbc) count, lymphocytes count and absolute lymphocytes count. Auto-haemanalyzer (Abacus junior hematology analyzer, S/no: 11759 made in Australia) was used to analyze all the haematological parameters [19].

Plasma Biochemical Parameters

Plasma biochemical parameteres were determined using plasma obtained following centrifugation of the anti-coagulant blood. The parameters include aspertate aminotransferase, alanine aminotransferase [20], glucose, creatinine, total protein [21], urea [22], bilirubin [23], sodium [24], bicarbonate ion [25], chloride ion [26] and potassium [27].

Histopathology

Rats that died during the study were subjected to post-morten examinations. Samples of organs and tissues (intestine, liver, kidney, heart, spleen and lung) were taken and fixed in 10% formalin solution. The tissues were thereafter embedded in paraffin wax and cut into 5µm thick and stained with haematoxylon and eosin (H&E) stain. Histopathological examination of the tissues using light microscope was thereafter carried out for presence of lesions [28].

RESULTS

Phytochemical Components

There was 2.91% yield from cold water extraction of Morinda lucida leaf. Phytochemical analysis revealed presence of tannins, glycosides, saponins, flavonoids, anthraquinones and reducing sugar whereas steroids were absent (Table **1**).

Table 1: Phytochemistry of the Crude Water Extract of Morinda Lucida Leaf

Test	Observations	Remarks
Tannins	Black precipitate formed	+ve
Glycosides	Black precipitate formed	+ve
Saponins	Frothing occurred	+ve
Flavonoids	Intense red colour	+ve
Steroids	No colour change	-ve
Anthraquinones	bright pink colour	+ve
Reducing sugar	brick red precipitate	+ve

Keys: + = present; - = absent

Proximate Components of *Morinda lucida* Leaf Powder

The proximate contents of *Morinda lucida* leaf powder are shown in Table **2**. Proximate analysis revealed moisture (8.88%), dry matter (91.12%), ash (8.71%), oil (25.16%), nitrogen free extract (34.30%), crude fibre (12.4%) and crude protein (10.50%).

Table 2: Proximate Composition (%) of *Morinda lucida* Leaf Powder

Component	Value/Result (percentage)
Dry matter	91.12%
Ash	8.71
Oil	25.16
Crude fibre	12.45
Crude protein	10.50
Moisture	8.88
Nitrogen free extract	34.30

Median Lethal Dose (LD_{50}) of *Morinda Lucida* Aqueous Leaf Extract

The limit dose of 2,000mg/kg did not cause death on the tested five rats. Similar situation was observed when the experimental rats were sequentially administered between 3,585 and 16,265mg/kg body weight. The toxicity signs observed are depression and lethargy. Hence LD_{50} is greater than 16,265mg/kg (Table **3**).

Chronic Toxicity Effects of *Morinda Lucida* Aqueous Leaf Extract

Haematological Parameters

Effect of prolonged administration of aqueous leaf extract of *Morinda lucida* on haematological parameters is presented in Table **4**. Treatment with the extract increased erythrocytes count significantly (P<0.05). Before treatment, the red blood cells count of the rats from control group was 4.18 ± 0.29 x 10^{12}/L. After the extract treatment, the red cells decreased significantly to 3.03 ± 0.63[a] in the group administered 16.26mg/kg body weight and increased to 4.94±0.44L[a] at dose level of 1626mg body weight respectively. The packed cell volume of groups administered 16.2mg/kg (21.00±2.39%[a]) and 162.6 mg/kg (23.42±1.07%[a]) decreased significantly (P<0.05) in comparison to the control group (25.61 ± 0.77%). However, the packed cell volume (27.49±0.85%[b]) increased significantly (p<0.05) in group administered 1,626.5mg/kg body weight as compared to the control group. Heamoglobin concentration significantly decreased (P<0.05) in the groups administered 16.26 and 162.65mg/kg and increased in the group administered 162.65mg/kg bodyweight respectively. The values of mean corpuscular volume of the control and experimental groups were comparatively similar. Mean corpuscular

Table 3: Toxicity Pattern of Aqueous Leaf Extract of *Morinda Lucida*

Dose (mg/kg)	Survival status	Toxicity effects
3585	0	Rapid and shallow respiration, lethargy
5170	0	Rapid and shallow respiration, lethargy
6755	0	Rapid and shallow respiration, lethargy
8340	0	Rapid and shallow respiration, lethargy
9925	0	Rapid and shallow respiration, lethargy
11510	0	Rapid and shallow respiration, lethargy
13095	0	Rapid and shallow respiration, lethargy
14600	0	Rapid and shallow respiration, lethargy
16465	0	Rapid and shallow respiration, lethargy

Key: 0 = survival

Table 4: Effects of Prolonged Administration of Varying Doses of Aqueous Leaf Extract of *Morinda lucida* on Haematological Parameters of *Rattus norvegicus*

Parameters	Experimental Groups			
	0.0mg/kg	16.26mg/kg	162.65mg/kg	1626.5mg/kg
Erythrocytes (x1012/L)	4.18 ±0.29	3.03 ± 0.63[a]	4.21 ±0.77	4.94 ± 0.44[b]
Packed Cell Volume (%)	25.61±0.77	21.00±2.39[a]	23.42 ±1.07[a]	27.49 ± 0.08[b]
Heamoglobin (g/L)	8.35 ±0.09	7.00 ± 0.93[a]	7.80 ± 0.56	9.16 ± 0.62[b]
Mean Corpuscular Volume (Fl)	58.91 ±1.22	58.95 ± 0.26	60.86 ± 0.82	58.24 ± 0.82
Mean Corpuscular heamoglobin (pg)	17.62 ± 0.17	18.61 ± 0.21	17.56 ±0.35	20.33 ±1.04[b]
Mean corpuscular heamoglobin concentration (g/L)	301.00 ±0.35	296.00 ±12.70	301.96 ±5.92	516.40 ± 49.37[b]
White blood cells count (x 109)	7.10 ±0.34	4.17±0.9[a]	6.85 ±1.06	4.44 + 0.39[a]
Absolute lymphocytes (x 109)	4.51 ±0.10	3.05 ± 0.72[a]	5.15 ±0.78	4.33 ± 0.52

Mean ± SEM of eight observations

a = significantly lower (P<0.05) in comparison with the control value

b = significantly higher (P<0.05) in comparison with the control value

heamoglobin (20.33±1.04pg[b]) and mean corpuscular heamoglobin concentration (516.40±49.37g/L[b]) significantly increased (P<0.05) in group administered 1626.5mg/kg as compared to the control group (Table **4**).

However white blood cells of the groups administered 16.26mg/kg (4.17±0.9[a]) and 1626.5mg/kg (4.44±0.39%[a]) decreased significantly (P<0.05) compared with that of the control group (7.10 ± 0.34%). Also, absolute lymphocytes (3.05±0.72%[a]) decreased significantly (P<0.05) compared with that of the control group (4.51 ± 0.10%) (Table **4**).

Biochemical Parameters

The effect of aqueous leaf extract of *Morinda lucida* on biochemical parameters of Rattus norvegicus is presented in Table **5**. Plasma aspartate aminotransferase (7.71±1.67µ g/L[a]) decreased significantly (P<0.05) in the group administered 16.26mg/kg body compared to the control group (12.38 ± 2.24µg/L). Alanine aminotransferase decreased significantly (P<0.05) in groups administered 16.26 and 1,626.5mg/kg given 16.55 ± 2.52 and 16.99 ± 1.99µg/L respectively as compared to the control group (21.10 ± 2.51µg/L). Glucose, creatinine, total protein, bilirubin and chloride ion of the groups administered 16.26,

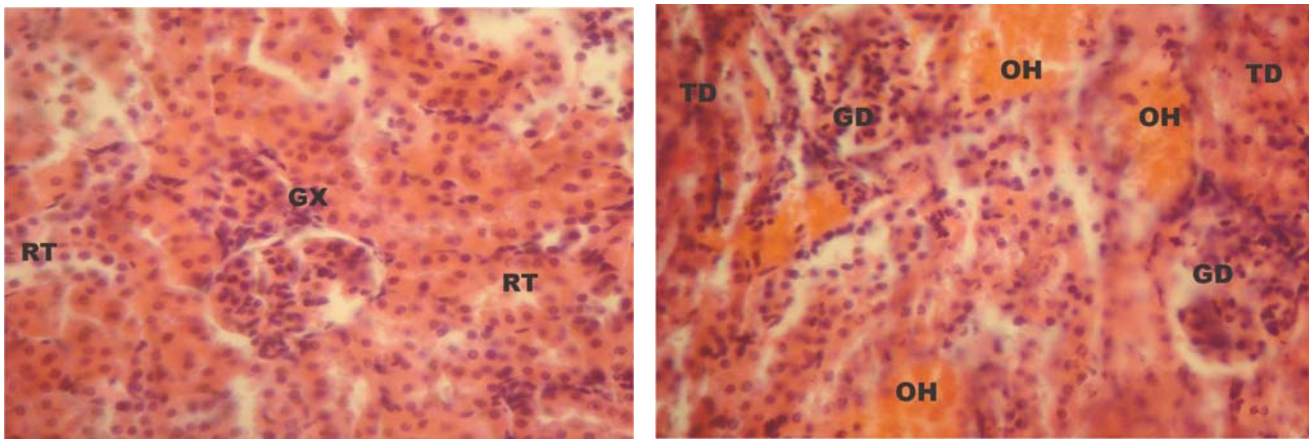
Table 5: Effects of Prolonged Administration of Varying Doses of Aqueous Leaf Extract of *Morinda lucida* on Biochemical Parameters of *Rattus norvegicus*

Parameters	Experimental Groups			
	A(0.0mg/kg)	B(16.26mg/kg)	C(162.65mg/kg)	D(1626.5mg/kg)
Aspartate aminotransferase (µµ/L)	12.38 ± 2.24	7.71 ± 1.67[a]	12.71 ± 1.52	11.86 ± 2.55
Alanine aminotranferase (µg/L)	21.10 ±2.51	16.55 ±2.52[a]	23. 66 ± 2.4[b]	16.99 ± 1.99[a]
Glucose (g/dL)	134.75 ±3.01	10.14±2.22[a]	18.86±1.71[a]	13.14±1.23[a]
Creatinine (mg/dL)	1.09 ±0.24	0.79±0.92[a]	0.68±0.16[a]	0.68±0.16[a]
Total protein (mmol/L)	7.56 ±1.97	2.67±1.01[a]	4.87±1.40[a]	4.77±0.91[a]
Urea (mmol/L)	2.83 ±0.89	3.06±0.60[b]	4.18±0.49[b]	3.3 ± 0.87[b]
Total bilirubin (mmol/L)	3.14 ±0.98	0.74±0.36[a]	2.59 ± 1.06[a]	2.22 ± 1.10[a]
Sodium (mmol/L)	131.62 ±5.59	95.96 ±11.03[a]	135.27 ± 5.91[b]	120.84 ±8.38[a]
Potassium (mmol/L)	3.74 ±0.46	2.90±0.26	3.31±0.25	3.93 ±0.52
Bicarbonate (mmol/L)	24.63±1.58	21.57±0.92[a]	22.71 ±2.86	20.80 ±1.96[a]
Chloride (mmol/L)	82.75 ±6.64	71.42±'5.43[a]	78.57 ±8.55[a]	76.57 ±5.93[a]

Mean ± SEM of eight observations

a = significantly lower (P<0.05) in comparison with the control value

b = significantly higher (P<0.05) in comparison with the control value



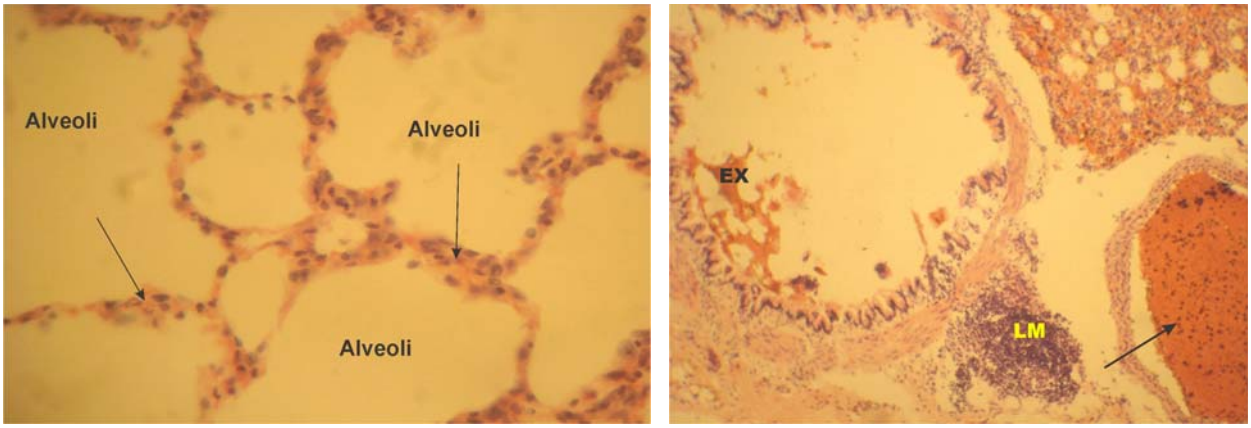
a **b**

Figure 1: a. Photomicrograph of kidney of control rat showing normal glomerulus (GX), and renal tubules (RT) of the cortex H&E x400. **b.** Photomicrograph of rat kidney treated with 1,626.5mg/kg body weight of *Morinda lucida* aqueous leaf extract for 28 days showing tubular degeneration (TD), glomerular degeneration (GD), multi-focal interstitial haemorrhage (O) H&E x400.

162.65 and 1,626.5mg/kg significantly decreased (P<0.05) in comparison with the control group. Sodium ion significantly decreased (P<0.05) in groups administered 16.26 and 1,626.5mg/kg body weight given 95.96 ± 11.03 and 120.84 ± 8.38 mmol/L respectively. However, the group administered 162.65mg/kg had significantly increased level of sodium ion given 135.27 ± 5.91mmol/l[a]. The bicarbonate ion was significantly decreased (P<0.05) in groups administered 16.26 and 1,626.5gmg/kg given 21.57 ± 0.92 and 20.80 ± 896mmol/L respectively in comparison with the control group (24.63±1.58mmol/L). Plasma potassium ion appeared to be same in both the extract treated and the control group of rats.

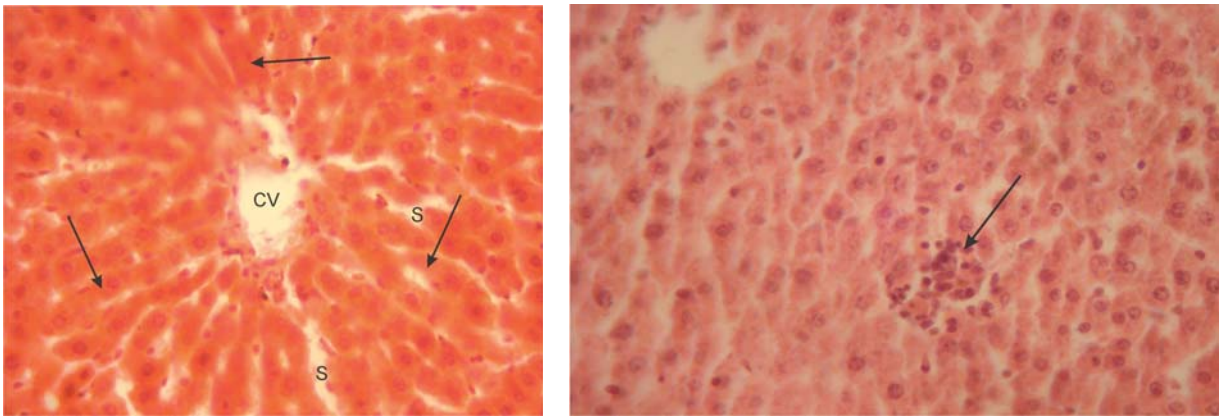
Histopathology

Kidney from control group of rat showed normal glomeruli and renal tubules in the cortex (Figure **1a**) whereas the micro-photography of kidney of rat treated with 1,626.5mg/kg body weight of aqueous leaf extract of *M. lucida* showed tubular degeneration, glomerular degeneration and multifocal interstial hemorrhage (Figure **1b**). Lung from the control group of rat showed normal alveoli and alveolar septae (Figure **2a**) but the lung of rat administrated 1,626.5mg/kg of the extract for 28 days revealed bronchiolar exudates, lymphocytic aggregation and congestion of blood (Figure **2b**). The liver of rat from control group showed normal central vein, sinusoids and normal hepatocytes (Figure **3a**). However, the rat administered 1,626.5mg/kg showed mild mononuclear cellular infiltration (Figure **3b**). The heart of a rat from the control group revealed normal cardiac muscle fibres (Figure **4a**) nevertheless, the heart of a rat administered 1626.5mg/kg showed multifocal and interstial haemorrhage and cloudy swelling of cardiac muscle (Figure **4b**). The spleen of a rat from the control group showed normal red pulp,



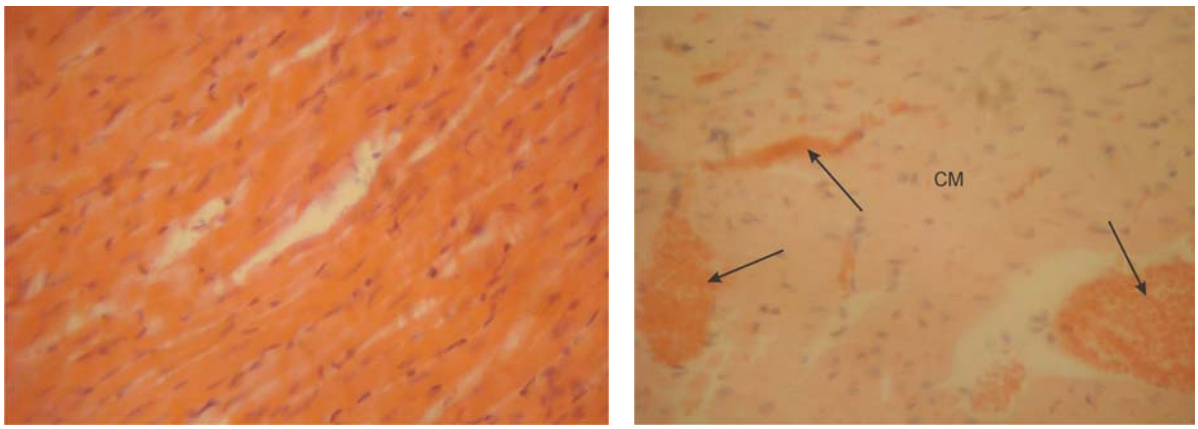
a **b**

Figure 2: a. Photomicrograph of lung of control rat showing normal alveoli and alveolar septae (arrows) H&E x400. **b.** Photomicrograph of rat lungs treated with 1,626.5mg/kg body weight of *Morinda lucida* aqueous leaf extract for 28 days showing mild bronchiolar exudates (EX), lymphocytic aggregation (LM) and congestion of blood (arrows) H&E x400.



a **b**

Figure 3: a. Photomicrograph of control rat liver showing normal central vein (CV), sinusoids (S) and normal hepatocytes radiating away from the central vein (arrows) H&E x400. **b.** Photomicrograph of liver of rat treated with 1,626.5mg/kg body weight of *Morinda lucida* aqueous leaf extract for 28 days showing mild mononuclear cells infiltration (arrows) H&E x400.



a b

Figure 4: a. Photomicrograph of heart of control rat showing normal cardiac muscle fibres H&E x400. **b.** Photomicrograph of heart muscle of rat treated with 1,626.5mg/kg body weight of *Morinda lucida* aqueous leaf extract for 28 days showing multi-focal and interstitial haemorrhage (arrows) and cloudy swelling of cardiac muscle (CM) H&E x400.

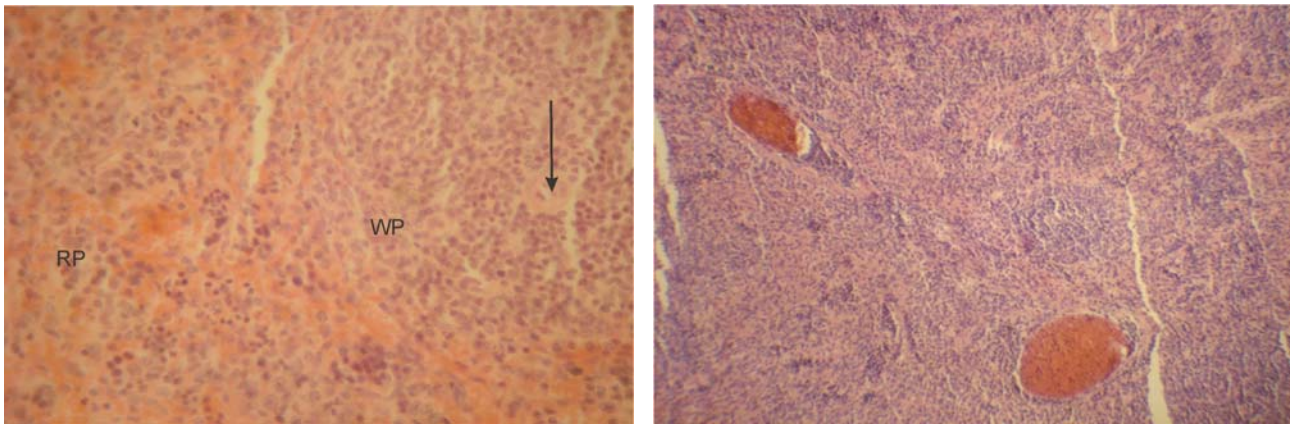
white pulp and eccentric arteriole (Figure **5a**). Whereas the spleen of a rat administered 1,626.5mg/kg body weight of the extract showed wide spread white pulp hyperplasia (Figure **5b**).

Statistical Analysis

The data on haematological and biochemical parameters were expressed as mean ± SEM of eight observations. Test for significance at 5% level of probability among the treatment groups was performed using one-way analysis of variance (ANOVA). Least significant difference was used to detect significant difference between the treatment and the control group as well as among treatment groups [29, 30].

DISCUSSION

The phytochemical screening of *Morinda lucida* leaf extract showed presence of useful chemical compounds such as glycosides, flavonoids, saponins, tannins, anthraquinones, and reducing sugar. The presence of flavonoids in the leaf of *Morinda lucida* may be responsible for its antibacterial, anti-inflammatory [7] and inhibitory effects on cancers [2]. Presence of glycosides in the aqueous leaf extract of *M. lucida* may be responsible for promoting gastric emptying. Saponins present in *M. lucida* may be responsible for anti-inflammatory activity [7] of the extract. The presence of tannin in the *M. lucida* may be responsible for its anti-hypertensive, antidysenteric and anti-ulcer activities [12]. But presence of anthraquinones may be responsible for the antimalarial activity [31]. However, the absence of steroids in our study disagrees with the report of Akinmoledun *et al.* [11] indicating that steroid is present in *M. lucida*. This different may be due to difference in geographical location of the plants. The 91.12% dry matter signifies that the plant was relatively dried and also, the plant



a b

Figure 5: a. Photomicrograph of spleen of control rat showing normal red pulp (RP), white pulp (WP) and eccentric arteriole (arrow) H&E x400. **b.** Photomicrograph of spleen of rat treated with 1,626.5mg/kg body weight of *Morinda lucida* aqueous leaf extract for 28 days showing wide spread white pulp hyperplasia H&E x200.

has high contents of both nutritional and anti-nutritional factors. WHO [32] reported that medicinal plant materials that contain excess water would encourage microbial growth, presence of fungi or insect and deterioration. The 8.7% nutritional content (Table **2**) of ash is suggestive of high content of micro and macro elements in the leaf of Morinda lucida. Our findings agree with the report of Ayodeji *et al.* [5] indicating that mercury, cadmium, arsenium and lead are present in *M. lucida*. However, 25.16% component of oil present in *M. lucida* may be suggestive of high content of terpenes, sesquiterpenes and other components that have wide range of therapeutic uses. Our findings agree with the report of Cimanga *et al.* [12] who indicated that the plant contains triterpenic acids; ursolic and oleanolic acids that exhibited both *in vitro* and *in vivo* anti plasmodial activities. Perhaps it is the presence of oil in *M. lucida* that gives the plant ability to heal several gastrointestinal problems. However the presence of nitrogen free extract (34.30%) and crude fibre (12.45%) in *M. lucida* leaf may be a clear indication of very high content of carbohydrate in the plant. This agrees with the report of McDonald *et al.* [17] that the main component of the dry matter of all the plant is carbohydrate. This may be because; cell walls of the plant store energy in form of carbohydrate such as starch and fructans. The 10.50% crude protein content of *M. Lucida* may suggest less quantity of protein in the plant leaf, despite the fact that crude protein is not a true protein.

The evaluation of the toxic effect of plant is indispensible in assessing its safety for both human and animal use. Acute toxicity study of the water extract of *M. lucida* leaf showed that the plant is safe. The extract did not cause death in the test rats administered between 2,000mg/kg and 1,626.5mg/kg of *M. lucida* aqueous leaf extract. However using toxicity rating of Hodge and Sterner scale, the plant is relatively harmless, but using the modified version of Hodge and Sterner rating the plant is practically non-toxic at the dose greater than 1,626.5mg/kg for a period of 14 days [33, 34]. But the administration of aqueous leaf extract of *M. lucida* to the rats, at various doses for 28 days have both beneficial and deleterious effects. The revealed significant decreased erythrocytes, packed cell volume, and haemoglobin in groups administered 16.26mg/kg and 162.65mg/kg (Table **3**) are suggestive of anaemic property of the extract. Ayodeji *et al.* [5] reported that *M. Lucida* contains lead and Cadmium, which can cause anemia in animal. Conversely, the increased packed cell volume and erythrocytes observed in the group administered 1,626.5mg/kg body weight may indicate antianaemic effect of the plant, probably due to presence of flavonoid. Substances with antianaemic effect are known to stimulate increased production of erythrocytes, improve the value of packed cell volume and haemoglobin [35]. However, the increase in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in groups administered 1,626.5mg/kg body weight may be due to inability of liver to metabolize haemoglobin. But since mean corpuscular volume is normal, hyperglobinaemia observed in our study may be due to other pathological condition. Because normocytic normochromic anaemia which is non-regenerative [36] is caused by haemolysis, chronic diseases, acute blood loss, renal disease, mixed deficiencies and bone marrow failure (e.g. post-chemotherapy, infiltration by carcinoma) [37]. However, the PCV value (25.61%) of the control group was lower in comparison with the value (34.5%) reported earlier by Saganuwan *et al.* [38]. This decreased PCV may be attributable to the use of haemanalyzer. Willard and Tvedten [39] reported that using machine to analyze haematological parameters can account for a shortfall in PCV value of 3.0% or more. Although Cheesbrough [19] reported that packed cell volume varies according to age, gender and altitude. But Tvedten and Weiss [37] reported that species difference could account for difference in packed cell volume. The decreased Leucopenia and lymphocytopenia observed in the extract treated rats may be caused by inflammation and stress respectively [40].

The results of biochemical study indicate that the administration of aqueous leaf extract of *Morinda lucida* for a period of 28 days has mild to moderate deleterious effects on some biochemical parameters investigated at dose levels between 16.26 and 1,626.5mg/kg body weight. The decreased level of alanine aminotransferase in group administered 16.26 - 1,626.5mg/kg bodyweight and aspartate amino-transferase in group administered 16.26mg/kg suggest that the liver is not under stress. Our findings agree with the report of Ojekale *et al.* [41] and Oduola *et al.* [42] that low levels of ALT and AST indicate that the liver is not under stress. But at dose level of 162.65mg/kg body, there was increased alanine aminotransferase which may suggest hepatic involvement. The significant decrease in plasma glucose levels of the rats treated 16.26 – 1,626.5mg/kg of the extract agrees with the report of Saganuwan [9] indicating that aqueous extract of *Morinda lucida* has potential anti-diabetic activity. The decrease levels of

creatinine observed in all the extract treated groups indicate metabolic effect of the extract on the body protein of the experimental rats. The turnover rate of body protein varies from one tissue to another and the tissues are replaced at intervals of hours, days or months. The energy of urine is present in nitrogen containing substance such as urea and creatine. Creatine of muscles is converted to creatinine which is excreted in urine [43] invariably decreasing plasma creatinine. Ganong [44] reported that muscles break down can lead to creatinuria which may in turn lead to hypocreatinemia. Creatinine, which diffuses throughout the body fluid, is formed from glycerine, arginine and methionine of guainodoacetate by s-adenysyl-methionine by methylation [45]. The increased plasma level of urea in all the extract treated groups may be due to nephritis as evidenced by decreased plasma total protein levels of all the experimental groups of rats administered different doses of the extract. Werner and Turnwald [46] reported that haemorrhage, exudation from severe skin lesions and protein-losing enteropathy can cause moderate to severe hypoproteinemia. Postmortem examination of the dead rats; one each from group administered 16.26 and 1,626.5mg/kg revealed gastroenteritis and congestion of the liver. Hypoproteinemia can be caused by draining wound, diarrhoea, dysentery, renal dysfunction, pregnancy, hepatic disorders, lactation and parasitism [21].

Decreased total bilirubin levels were observed in the extract treated rats at 28th day of the treatment. This decrease may indicate that the liver had no severe lesions that would prevent the excretion of bilirubin through the bile duct. The hypochloremia and hyponatremia observed in all the extract treated groups may be due to diarrhea or kidney disease problem caused by the extract. But hypochloremia due to increased renal chloride excretion is a normal adaptation to chronic respiratory acidosis. Persistent hypochloremia is an indication of a need to determine serum sodium, potassium and total carbon dioxide concentrations with or without blood gas analysis [47]. Plasma bicarbonate ion was increased in all the experimental rats suggesting acidotic nature of the plasma. But plasma potassium level of the experimental rats is comparable to that of the control rats. Hence, our findings disagree with the report of Oduola *et al.* [42] indicating that ingestion of *Morinda lucida* leaf extract has no toxic effect on liver and kidney functions. The differences between our findings and those of Oduola *et al.* [42] may be due to the following factors; the nature of the soil on which the plants were grown, the strains of the rats used for the studies, general laboratory practice, standard operating procedures, climatic factors such as ambient temperature, humidity and pH of soil.

The observed kidney damage as evidenced by increased urea in rats administered 1,626.5mg/kg in our study agrees with the report of Saganuwan and Onyeyili [48] indicating that plant extract can cause kidney damage. The lesion observed in the lung might be responsible for respiratory depression observed in our study. However, lymphocytic aggregation of mononuclear cells in pneumocytes and hepatocytes and wide spread hyperplasia of white pulp may be responsible for lymphopenia and leucopenia observed in the study. White pulps comprise of white blood cells that are manufactured similar to those manufactured in the lymphnodes. They are part of body immune systems [46]. Fange and Sunde [49] reported that lymphocytopoeisis takes place in the spleen, the cranial lymphomyeloid tissue, the thymus, and possibly in other less well defined lymphomyeloid structures. White pulp comprise of white blood cells that are manufactured similar to those manufactured in the lymphnodes. They are part of body immune systems [50]. Saganuwan and Onyeyili [48] had earlier reported that plant extract can cause mononuclear cellular infiltration. Mild mononuclear cells infiltration as well as cloudy swelling and multifocal interstial hemorrhage of cardiac muscle seen in the experimental rats are suggestive of toxic organ involvement at high dose level of 1,626.5mg/kg. However, normal glomeruli and renal tubules in the cortex of kidney, normal alveolar and alveolar septae of lung, normal central vein, sinusoids and hepatocytes of liver, normal cardiac muscle fibres of the heart and normal red pulp and white pulp of the control rats show that the rats used for our experiment were healthy prior to period of experimentation.

Conclusively, aqueous leaf extract of *M. lucida* contained tannins, glycosides, saponins, flavonoids, anthraquinones and reducing sugars. Proximate analysis of *M. lucida* leaf powder revealed moisture (8.88%), dry matter (91.12%), ash (8.71%), oil (25.16%), crude fibre (12.45%), crude protein (10.50%) and nitrogen free extract (34.30%). Aqueous leaf extract of *Morinda lucida* caused increased erythrocytes, PCV, hypoglobinemia, leucopenia and lymphopenia at dose levels of 16.26mg/kg and 162.65mg/kg body weight. At dose level of 1,626.5mg/kg body weight, the extract caused decreased AST and ALT,

hypoglycemia, hypocreatinemia, hypoproteinemia, hyperuremia, hypo and hyper bilirubinemia, hyponatremia, hypochloremia, decreased bicarbonate ion and hyperuremia.

REFERENCES

[1] Burkill, H.M. 1984. The useful plants of West Africa, Vol. 4.

[2] Zinudzi, C. & Cardon, D. 2005. Plant Resources of Tropical Africa/Resources. WYYP: / database. Prota.org/search.

[3] Koumaglo, K., Gbeassor, M., Nikabu, O., De Souza, C. & Werner, W. 1992. Effects of three compounds extracted from *Morinda lucida* on Plasmodium falciparum. Planta Medica 58(6): 533-534. http://dx.doi.org/10.1055/s-2006-961543

[4] Elias, S.O., Ladipo, C.O., Oduwole, B.P., Emeka, P.M., Ojobor, P.D. & Sofola, O.A. 2007. *Morinda Lucida* reduces contractility of isolated uterine smooth muscle of pregnant and non-pregnant mice. Nigerian Journal of Physiological Science 22(1-2): 129-134.

[5] Ayodeji, F.B., Olorunsola, O.E. & Olukemi, O.D. 2010. Accumulation of some metals in *Morinda lucida* from Nigeria. Toxicology Environmental Chemistry 92(9): 1665-1672. http://dx.doi.org/10.1080/02772248.2010.495074

[6] Asuzu, I.U. & Chineme, C.N. 1990. Effect of Morinda leaf extract on Trypanosoma brucei infection in mice. Journal of Ethnopharmacology 30(3): 307-312. http://dx.doi.org/10.1016/0378-8741(90)90109-7

[7] Tona, L, Ngimbi, N.P., Tsakala, M., Mesia, K., Cimanga, K., Apers, De Bruyne T., Peiters, L., Totte, J. & Viictinck, A.J. 1999. Anti-malarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasha, Congo Journal of Ethnopharmacology 68 (1-3): 293-203.

[8] Raji, Y., Akinsomisoye, O.S. & Salman, T.M. 2005. Antispermatogenic Activity of *Morinda lucida* in male Rats. Asia Journal of Andrology 7. (4): 405. http://dx.doi.org/10.1111/j.1745-7262.2005.00051.x

[9] Saganuwan, S.A. 2009. Tropical plants with antihypertensive, antiasthmatic, and antidiabetic value. Journal of Herbs, Spices and Medicinal Plants 15: 24-44. http://dx.doi.org/10.1080/10496470902787477

[10] Hounzangbe-Adote, S., Fouraste, I., Moutairous, K. & Hoste, H. 2007. In vitro effects of four tropical plants on the activity and development of the parasitic nematode, Trichostrongylus colubriformis. Journal of Helminthology 79: 29-33.

[11] Akinmoladun, A.C., Obuotor, E.M. & Farombi, E.O. 2010. Evaluation of antioxidant and free radical scavenging capacities of some Nigeria indigenous medicinal plants. Journal of Medicinal Food 13(2): 444-451. http://dx.doi.org/10.1089/jmf.2008.0292

[12] Cimanga, R.K., Tona, G.L., Mesia, G.K., Kambu, O.K., Bakana, D.P., Kalenda, P.D.T., Penge, A.O., Muyembe, J.J.T., Totte, J., Pieters, L. & Vietinck, A.J. 2006. Bioassay – guided isolation of antimalarial triterpenoid acids from the leaves of Morinda lucida. Journal of Medicinal Plants 4(9): 677-681.

[13] Council for International Organization of Medical Sciences (CIOMS) 1985. International Guiding Principles for Biomedical Research Involving Animals C/o WHO 121, Geneva 9.

[14] Ajagbonna, O.P., Esaigun, P.E., Alayande, N.O. & Akinloye, A.O. 2002. Anti-malarial activity and haematological effect of stem bark water extract of *Nudealatifolia. Bioscience Research Communication* 14 (5): 481-486.

[15] Brain, K.R. & Turner, T.D. 1975. *The practical Evaluation of phyto-pharmaceuticals,* Wright-Scientica, Bristol, 1975; 81-86.

[16] AOAC. 1984. *Official Methods of Analysis*, 12th ed., Washington DC pp. 129 – 133.

[17] Mcdonald, P., Edwards. R.A., Greenhalgh, J.F.D. & Morgan, C.A. 1998. Animal Nutrition, 5th edition, Pearson Education ltd., Edinburgh UK, 607.

[18] Yamba, O., Innocent, P.G. & Odile, G.N. 2007. Biological and toxicological study of aqueous root-extract from *Mitragyna inermis* (Willd Oktze) Rubiaceae. *International Journal of Pharmacology* 3(1): 80-85. http://dx.doi.org/10.3923/ijp.2007.80.85

[19] Cheesbrough, M. 2004. District Laboratory Practice in Tropical Countries Part 2, Cambridge low price edition, Cambridge University press, 434pp.

[20] Tietz, N.N. 1986. *Textbook of Clinical Chemistry,* W.B. Saunder Company, Philadelphia, pp1187.

[21] Chauchan, R.S. & Agarwal, D.K. 2006. *Textbook of Veterinary Clinical and Laboratory Diagnosis*, Second edition, Jaypee Brothers Medical Publisher (p) Ltd., New Delhi, India, pp193-207.

[22] Fawcett, J.K. & Scott, J.E. 1960. *Determination of Serum urea. Clinical pathology* 13:156.

[23] Novros, V.S., Koch, T.R. & Knoblock, E.C. 1978. *Clinical Chem.* 25:1891.

[24] Tietz, N.W. 1974. *Textbook of Clinical Chemistry*, 2nd ed., W.B. Saunders Company, Philadelphia, PA, 703.

[25] Sacks, D.B. 1999. Carbohydrates. In: Burtis, C. A. and Ashwood, E. R. (editors). *Tietz Textbook of Clinical Chemistry* 3rd ed., W. B. Saunders Company, Philadelphia, 750-808.

[26] WHO. 1987. *Methods Recommended for Essential Chemical and Haematological Tests for Intermediate Hospital Laboratories,* pp1-92.

[27] Ray Sarkar, B.C. & Chauchan, U.P.S. 1967. Determination of Plasma Potassium ion. *Anal Biochemistry*. 20:155.

[28] Carlton, H.M. 1980. Carlton' Histological Techniques, 5th ed. Edinburgh pp. 200.

[29] Olayinka, A.I., Taiwo, V.O., Raji-Oyelade, & Farai, I.P. 2005. Methodology of Basic and Applied Research, Ibadan, Nigeria pp. 200.

[30] Zar, J.H. 2008. Biostatistical Analysis, 4th ed., New Delhi Pearson Education pp. 663.

[31] Makinde, J. & Obih, P.O. 1985. Screening of *Morinda lucida* leaf Extract for Morinda action on plasmodium berghei in mice. African Journal Medicine and Medical Science 14(1-2): 59-63.

[32] WHO. 1998. Quality Control Method for Medicinal Plants Materials, Geneva, Switzerland pp. 115.

[33] Garg, S.K. 2000. Veterinary Toxicology, CBS Publishers and Distributors, Darya Ganj, New Delhi, India pp. 9.

[34] Hodge, H.C. & Sterner, J.H. 1949. Toxicity rating. American Industrial Hygiene Association Quarterly 10(4): 93.

[35] Brown, B.A. 1976. *Haematology principles and procedures*, 2nd ed. Lea and Febiger, Philadelphia, pp. 56 – 81.

[36] Tvedten, H. & Weiss, D. 1999. Erythrocyte disorders. In: Willard, M.D., Tvedten, H. and Turnwald, G.H. (eds). Small Animal Clinical Diagnosis by Laboratory Methods, W.B. Saunders Company, Philadelphia London Toronto 395.

[37] Hoffbrand, A.V., Moss, P.A.H. & Pettit, J.E. 2006. Essential Haematology, 5th edition, Blackwell publishing 380.

[38] Saganuwan, S.A., Ahur, V.M. & Mhomga, L.I. 2010. Sodium chloride increase body weight of non-water deprived Rattus norvegocus. Aust. J. Basic & Appl. Sci. 4(8): 3224-3229.

[39] Willard, M.D. & Tvedten, D.C. 1999. Gastrointestinal, Pancreatic and peptic disorders PP 172-207. In: *Small Animal Clinical Diagnosis by Laboratory Methods* (Willard, M.D., Tvedten, H. and Turnwald, G.H. editors), 3rd edition, W.B. Saunders Company, Philadelphia, USA, 395.

[40] Latimer, K.S. & Tvedten, H. 1999. Leukocyte disorders. In: Willard, M.D., Tvedten, H., Turnwald, G.H. Small Animal Clinical Diagnosis by Laboratory Methods, 3rd ed., W.B. Saunders Company, Philadelphia London Toronto 395.

[41] Ojekale, A.B., Ojiako, O.A., Saibu, G.M., Lala, A. & Olodude, O.A. 2007. Long-term effects of aqueous stem bark extract of Cissus populnea (Guill. and Per.) on some biochemical parameters in normal rabbits. Afr. J. Biotechnol 6(3): 247-251.

[42] Oduola, T., Bello, I., Adeosun, G., Abdul-Waheed, A., Raheem, G. & Avioworo, G. 2010. Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to *Morinda lucida* leaf extract. North American Journal of Medical Science 2(5): 230-233.

[43] Rodwell, V.W. 2003. Conversion of amino acids to specialized products In: Harper's Illustrated Biochemistry, 26th ed., McGraw Hill, London 302-345.

[44] Ganong, W.F. 2001. Creatine and creatinine. In: Review of Medical Physiology, 21st ed., McGraw Hill, London, UK 298.

[45] Barsanti, J.A., Lees, G.E., Wllard, M.D. & Green, R.A. 1999. Urinary disorders In: Small Animal Clinical Diagnosis By Laboratory Methods, W.B. Saunders Company, Philadelphia London Toronto 395.

[46] Werner, L.L. & Turnwald, G.H. 1999. Immunologic and plasma protein disorders In: Willard, M.D., Tvedten, H. and Turnwald, G.H. (eds). Small Animal Clinical Diagnosis by Laboratory Methods, W.B. Saunders Company, Philadelphia London Toronto 395.

[47] Di Bartola, S.P., Green, R.A., D.E., Norais, H.S.A. & Willard, M.D. 1999. Electrolyte and acid-base disorders. In: Willard, M.D., Tvedten, H., Turnwald, G.H. Small Animal Clinical Diagnosis by Laboratory Methods, 3rd ed., W.B. Saunders Company, Philadelphia London Toronto 395.

[48] Saganuwan, S.A. & Onyeyili, P.A. 2010. Biochemical effects of aqueous leaf extract of abrus precatoruis (Jecquirity bean) in Swiss albino mice. Herba Polonica 56(3): 63-80.

[49] Fange, R. & Sundell, G. Lymphomyeloid tissue, blood cells and plasma proteins in Chimaera monstrosa (Piscea, Holocephali). Acta Zoologica 1969; 50: 155. http://dx.doi.org/10.1111/j.1463-6395.1969.tb00537.x

[50] Guyton, A.C. & Hall, J.E. 2009. Textbook of Medicinal Physiology, 11th edition, WB Saunders, An Imprint of Elsevier, Philadelphia pp. 116.

Received on 28-10-14 Accepted on 11-02-14 Published on 23-04-2014