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Sub-acute and chronic toxicity profiles of *Markhamia tomentosa* ethanolic leaf extract in rats

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ABSTRACT

Ethnopharmacological relevance: *Markhamia tomentosa* (Benth.) K. Schum Ex Engl. (Bignoniaceae) is used in traditional African medicine for the treatment of diarrhoea, oedema, pain and malaria. The leaf extract was reported to show no visible sign of toxicity on acute exposure. This present study investigates the sub-acute and chronic toxicity effects of *Markhamia tomentosa* in rats.

Materials and methods: The animals (n=6/group) were treated daily with the extract at doses of 40, 200 and 1000 mg/kg orally for 28 and 90 days. Control rats received distilled water and all animals were weighed at 7 days interval. The haematological, biochemical and histological parameters were determined.

Results: The extract showed non-significant changes in body weight gain of treated compared to control rats in both studies. Extract significantly decreased red blood cell (RBC), mean cell haemoglobin concentration and increased mean corpuscular volume (MCV) parameters after the 28 day study. In the 90 day study, a significant increase in white blood cell, RBC, platelets and decrease in MCV and mean cell haemoglobin (MCH) parameters were observed. Biochemical parameters were significantly changed in both studies; triglycerides, total protein, alanine transaminase, aspartate transaminase and albumin showed significant increase while creatinine, blood urea nitrogen and uric acid levels showed significant decrease. Significant increase in liver weight with no treatment-related histological changes was observed in all harvested vital organs.

Conclusion: *Markhamia tomentosa* extract elicited non-toxic effect in the liver and kidney function parameters in rats. Thus, the extract is safe when administered orally.

1. Introduction

The use of medicinal plants for health and disease management is probably the oldest existing method that humanity has used to cope with illness. Being an important aspect of various traditional medicine systems, medicinal plants have been used therapeutically all around the world. Although all the systems of traditional medicine, from Unani to Tibetan medicine; Ayurveda to Chinese traditional medicine; Amazonian to African traditional medicine are based on different theoretical and cultural differences, they all integrate phytotherapy in their doctrine (WHO, 2007).

Despite the growing global demand of herbal medicine, there are still concerns associated with not only their use, but their safety (Obidike and Salawu, 2013). Herbal remedies are generally referred to as safe and are presented to the public as being "natural" and completely "safe" due to their long history of use (Adewunmi and Ojewole, 2004; Adeyemi et al., 2010; Afolabi et al., 2012). Nonetheless, the growing number of herbal product users around the globe and lack of scientific data on the safety profile of herbal products make it necessary to conduct toxicity study of herbal products. Although there has been reported increase in the ethnopharmacological investigations of African medicinal plants in literature, most of the plants have not undergone exhaustive toxicological tests such as are required for modern pharmaceutical compounds (Watt and Breyer-Brandwijk, 1962; van Wyk et al., 1997, 2000).

Markhamia tomentosa (Benth) K. Schum. ex. Engl. locally known by the Yoruba people of Southwest Nigeria as "oruru" is a tree

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belonging to the family Bignoniaceae. It is found mostly in West African countries from Senegal, Ghana, Nigeria to Cameroun extending southward Congo and Angola (Temdie et al., 2012).

In traditional African medicine (TAM), the leaves are used in the treatment of diarrhoea, scrotal elephantiasis and as an antidote for snake venom (Irvine, 1961; Burkill, 1985). The leaf decoction and chewed leaves are used for the treatment of general body pains (Burkill, 1985; Aladesanmi et al., 2007). The decoction of the leaves and bark are used as laxative while the stem bark is used in the treatment of malarial and intercostal pain (Adjanohoun et al., 1996; Tantangmo et al., 2010). This species has also found use in ethnoveterinary medicine as its leaves and roots are used in the treatment of diarrhoea, dysentery, fever, pain and inflammation in animals (Irvine, 1961; Borokini and Omotayo, 2012).

In terms of scientific evaluation, several *in-vitro* and *in-vivo* pharmacological investigations have reported the antimicrobial, antioxidant, antiplasmodial, antialzeheimer, antilarvicidal, analgesic, anti-inflammatory and antiulcer activities of *Markhamia tomentosa* (Aladesanmi et al., 2007; Tantangmo et al., 2010; Elufioye et al., 2010; Adebajo et al., 2012; Temdie et al., 2012; Sowemimo et al., 2013; Sofidiya et al., 2014).

In our earlier studies, we reported the anti-proliferative and underlying mechanisms of the leaf extract of the plant on brine shrimp larvae, HeLa and MCF-7 cancer cell lines as well as on Vero non-cancerous cell lines (Ibrahim et al., 2013). The chromosomal aberrations induction of the plant on *Allium* root cells was also reported (Ibrahim et al., 2014). Isolation, identification and characterisation of the pharmacological active compounds from *Markhamia tomentosa* are on-going.

Due to the wide application and the tendency of prolonged intake of this plant species, this study was therefore designed to investigate the dose and time-dependent chronic toxicity effects of *Markhamia tomentosa* in rats.

2. Materials and methods

2.1. Plant material and preparation of plant extract

The leaves of *Markhamia tomentosa* (Benth) K. Schum ex Engl. were collected from Oke-Igbo in Ondo state, Nigeria in February 2015. Taxonomic identification and authentication were carried out in the Herbarium of Department of Botany and Microbiology, University of Lagos where a voucher specimen (LUH 5535) was deposited. The leaves were air dried at room temperature (23 °C ± 2 °C) and pulverised in a mechanical grinder. Five kilogram of the dried leaf material was macerated in 50 L of absolute ethanol for 72 h at room temperature. The ethanol extract was filtered through a Whatman filter paper and evaporated to dryness under vacuum on a rotary evaporator (Buchi, Switzerland) at 40 °C to yield 13.31% (w/w). The dried extract was stored in the refrigerator at 4 °C until further use. For administration to experimental animals, the extract was resuspended in distilled water. The suspension was freshly prepared on a daily basis.

2.2. Experimental animals

Male and female Albino Wistar rats, weighing 110–130 g, were bought from the Laboratory Animal Centre of the National Agency for Food and Drug Administration and Control (NAFDAC), Yaba, Lagos, Nigeria. The animals were kept for a minimum of 7-days prior to oral administration of the extract at the Animal house of College of Medicine, University of Lagos, Nigeria to allow for their acclimatisation to the laboratory conditions. The animal room was ventilated with 12-h cycle of day and night light conditions at a temperature of 23 °C ± 2 °C and the animals were fed with standard rodent diet (Livestock Feeds PLC, Ibadan, Oyo state, Nigeria) and water *ad libitum*. The cage beddings and water bottles were cleaned on a daily basis.

The research protocols used in this study were in accordance with the requirements of the Research Grants and Experimentation Ethics Committee of the College of Medicine, University of Lagos, Nigeria (RGEEC/25/2015).

2.3. Sub-acute and chronic studies

A total of forty-eight rats were randomly divided into 4 groups of 6 male and 6 female rats per group for the toxicological studies. Six rats each (3 male and 3 female) were designated for the sub-acute and chronic toxicity studies. The rats were grouped based on three different treatment doses of the plant extract with one control group per study. The animals were daily treated *p.o.* with distilled water (control) and *Markhamia tomentosa* leaf extract at doses of 40, 200 and 1000 mg/kg for 28 and 90 days. The treatment doses representing one-fifth of the pharmacologically active dose, the pharmacologically active dose and five times the pharmacologically active dose respectively (Yemitan and Adeyemi, 2004; Afolabi et al., 2012). The pharmacological active dose was the most effective dose recorded in the investigation of the anti-inflammatory activity of *Markhamia tomentosa* leaf extract in rats (Sowemimo et al., 2013).

At the end of 28 and 90 d treatment periods, the rats were fasted of feed but left with drinking water *ad libitum* for 24 h and were sacrificed by decapitation under inhaled diethyl ether anaesthesia. Blood samples were collected from rats by retro-orbital puncture using capillary tubes into heparinised and non-heparinised centrifuge tubes for the haematological and biochemical studies respectively.

A deep longitudinal incision was made into the ventral surface of the abdomen and thorax of the sacrificed rats and by blunt dissection of the muscles and fasciae, vital organs such as liver, kidneys, heart, brain, testes, ovaries, spleen and lungs were exposed and harvested.

2.4. Measurement of body and organ weights

Throughout the experimental study, the animals were weighed weekly and the % weight change for each animal at the end of each study was calculated as given below:

% Weight change = (Difference between interval body weight and initial body weight ÷ initial body weight) × 100.

The weight of each harvested organ was standardized for 100 g body weight of individual rat.

2.5. Haematological analysis

Blood sample collected in the heparinised centrifuge tubes was analysed using an automated haematology analyzer (BC-3200). Parameters evaluated include white blood cell (WBC) count, haemoglobin (Hb), red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), haematocrit (HCT) and platelet count (PLT).

2.6. Serum biochemical analysis

Blood serum for the biochemical analysis was obtained by coagulation and centrifugation of the blood sample in the non-heparinized centrifuge tubes. Serum samples were analysed for alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), albumin (ALB), total bilirubin (TBIL), lipids (triglycerides (TG), total cholesterol (TCHO), high-density

lipoprotein (HDL-c)), total protein (TP), creatinine (Cr), blood urea nitrogen (BUN) and uric acid (URIC) using standard diagnostic test kits (Randox Laboratories Limited, Crumlin, County Antrim, BT29 4QY, UK) on automated Clinical System (Beckman Coulter Inc. Galway, Ireland). Serum electrolytes such as sodium (Na^+), potassium (K^+), chloride (Cl^-) Calcium (Ca^{2+}) and bicarbonate (HCO_3^-) were also determined.

2.7. Histopathological assessment

The harvested vital organs were weighed and fixed in 10% buffered formalin solution in labelled bottles to observe for possible histopathology changes. Following fixation, the tissues were exposed to routine processing, embedded in paraffin and section at 3–5 μm. Tissue sections were stained with hematoxyllin and eosin stain using Leica DM 500 microscope attached with camera Leica ICC50 HD (Leica Microsystems Ltd., Switzerland).

2.8. Statistic analysis

Results are presented as mean ± SEM for change in body and relative organ weights while data obtained for haematological and biochemical analysis are expressed as mean ± SD. The data were assessed by one-way analysis of variance (ANOVA) followed by Dunnett's post tests using GraphPad Prism® software. Level of significance was considered at values of $p < 0.05$.

3. Results

3.1. Effect of plant extract on body weight in the sub-acute and chronic studies

Table 1 showed that the plant extract compared to the control, caused non-significant ($p > 0.05$) increase in the percentage body weight in a non-dose-dependent manner with the highest increase recorded at the dose of 40 and 200 mg/kg in the 28 and 90 day treatment period respectively.

3.2. Effect of plant extract on organ weight in the sub-acute and chronic studies

As shown in Table 2, there was no significant ($p > 0.05$) difference in the weight of organs between the control and the treatment groups except for a significant non-dose-dependent increase in the weight of the lungs at the dose of 200 mg/kg ($p < 0.01$) and 1000 mg/kg ($p < 0.05$) in the 28 day treatment period.

The 90 day treatment period showed a significant ($p < 0.05$, 0.01) non-dose-dependent increase in weight of the liver and testes (Table 2) with the highest increase at the dose of 40 mg/kg ($p < 0.01$). A significant ($p < 0.05$) decrease in weight of the brain observed in this treatment period was also not dose dependent with the highest reduction recorded at 40 mg/kg dose.

Table 1
Effect of leaf extract on change in animal weight in the sub-acute and chronic studies.

Study	Dose (mg/kg)	Percentage weight change (%)
Sub-acute (28 days)	Control	23.33 ± 3.45
	40	32.67 ± 3.29
	200	22.33 ± 5.45
	1000	24.00 ± 7.97
Chronic (90 days)	Control	68.67 ± 5.81
	40	77.00 ± 6.67
	200	83.00 ± 8.59
	1000	73.67 ± 5.25

Values are mean ± SEM (n=6). No statistical difference ($p > 0.05$) between treated and control rats (One-way ANOVA followed by Dunnet's test).

Table 2
Effect of leaf extract on organ weights (per 100 g body weight) in the sub-acute and chronic study.

Study	Organ	Dose (mg/kg)			
		Control	40	200	1000
Sub-acute (28 days)	Heart	0.46 ± 0.04	0.39 ± 0.05	0.43 ± 0.04	0.43 ± 0.05
	Lungs	0.81 ± 0.05	0.99 ± 0.15	1.08 ± 0.07**	1.03 ± 0.03*
	Kidneys	0.68 ± 0.03	0.67 ± 0.13	0.70 ± 0.02	0.60 ± 0.02
	Spleen	0.45 ± 0.04	0.49 ± 0.03	0.44 ± 0.05	0.48 ± 0.04
	Liver	3.27 ± 0.36	3.81 ± 0.62	3.25 ± 0.19	3.93 ± 0.16
	Brain	1.19 ± 0.09	1.01 ± 0.22	1.13 ± 0.06	1.20 ± 0.11
	Testes	1.61 ± 0.05	1.56 ± 0.11	1.51 ± 0.08	1.31 ± 0.01
Chronic (90 days)	Heart	0.37 ± 0.09	0.36 ± 0.05	0.38 ± 0.03	0.39 ± 0.05
	Lungs	0.96 ± 0.22	0.81 ± 0.06	0.82 ± 0.07	0.83 ± 0.03
	Kidneys	0.55 ± 0.07	0.60 ± 0.05	0.62 ± 0.02	0.55 ± 0.02
	Spleen	0.35 ± 0.04	0.33 ± 0.01	0.32 ± 0.05	0.32 ± 0.04
	Liver	3.10 ± 0.12	3.88 ± 0.25**	3.63 ± 0.18*	3.75 ± 0.16*
	Brain	0.93 ± 0.05	0.73 ± 0.09*	0.74 ± 0.06*	0.75 ± 0.11*
	Testes	0.75 ± 0.11	1.31 ± 0.05**	1.13 ± 0.08*	1.29 ± 0.01**

Values are mean ± SEM (n=6).
* $p < 0.05$.
** $p < 0.01$ significantly different from control (One-way ANOVA followed by Dunnet's test).

3.3. Effect of plant extract on haematological parameters in the 28 and 90 days studies

The results presented in Table 3 showed that three parameters were statistically significant during the 28 d treatment period. A significant ($p < 0.05$, 0.01) reduction of RBC count at the dose of 1000 mg/kg and MCHC at 40 mg/kg and 200 mg/kg were observed in the treated rats. In addition, a significant ($p < 0.05$, 0.0001) non-dose-dependent increase in MCV with the highest increase at the dose of 40 mg/kg was observed during this treatment period. Other haematological parameters (WBC, Hb, MCH, HCT and PLT) of the treated rats were not significantly different when compared to the control rats.

Repeated oral treatment with the plant extract for 90 days exhibited a significant ($p < 0.05$, 0.01 and 0.0001) dose-dependent increase in WBC and PLT counts and a significant ($p < 0.05$, 0.01) non-dose-dependent increase in RBC at 200 mg/kg and 1000 mg/kg compared to control (Table 3). A significant ($p < 0.05$) non-dose-dependent decrease in MCV and MCH at 200 mg/kg compared to control was also observed in this treatment period. Other haematological parameters (Hb, MCHC and HCT) of the treated rats were not significantly different compared to the control rats.

3.4. Effect of plant extract on serum biochemical parameters in the 28 and 90 days studies

Table 4 shows the values of serum biochemical parameters after the 28 and 90 days treatment periods. Compared with the control rats, all measured serum biochemical parameters were not significantly ($p > 0.05$) different, except for a significant ($p < 0.05$) non-dose-dependent increase of plasma TG at the dose of 200 mg/kg and TP at the dose of 200 mg/kg and 1000 mg/kg in treated rats of the 28 day treatment period.

However, the serum levels of AST, ALT and ALB of treated rats in the 90 day treatment period showed a significant ($p < 0.05$, 0.01, 0.001, 0.0001) non-dose-dependent increase compared to control

Table 3
Effect of *Markhamia tomemtosa* leaf extract on haematological parameters in rats in the sub-acute and chronic toxicity studies.

Study	Parameter	Dose (mg/kg)			
		Control	40	200	1000
Sub-acute (28 days)	White blood cell ($\times 10^9$/L)	6.12 ± 0.71	6.75 ± 0.24	7.72 ± 1.01	5.83 ± 0.88
	Haemoglobin (g/L)	12.17 ± 0.61	10.98 ± 0.40	11.33 ± 0.57	10.27 ± 0.86
	Red blood cell ($\times 10^{12}$/L)	6.77 ± 0.22	5.97 ± 0.16	6.59 ± 0.21	5.60 ± 0.47*
	Mean corpuscular volume (fL)	55.3 ± 0.82	65.08 ± 1.07****	60.47 ± 1.69*	58.65 ± 1.21
	Mean cell haemoglobin (pg)	17.88 ± 0.43	18.33 ± 0.29	17.17 ± 0.70	18.35 ± 0.47
	Mean cell haemoglobin concentration (g/dl)	32.42 ± 0.46	28.23 ± 0.35**	28.42 ± 0.69*	31.50 ± 1.38
	Hematocrit (%)	37.4 ± 1.50	38.8 ± 1.23	39.72 ± 1.41	32.78 ± 2.80
	Platelet ($\times 10^9$/L)	646.17 ± 42.27	534 ± 38.11	633.17 ± 67.37	508.50 ± 76.89
Chronic (90 days)	White blood cell ($\times 10^9$/L)	6.95 ± 0.55	8.97 ± 0.27	12.33 ± 0.41****	12.78 ± 0.95****
	Haemoglobin (g/L)	14.05 ± 0.82	14.85 ± 0.61	15.83 ± 0.39	15.85 ± 0.34
	Red blood cell ($\times 10^{12}$/L)	7.04 ± 0.41	7.68 ± 0.29	8.54 ± 0.13**	8.23 ± 0.21*
	Mean corpuscular volume (fL)	58.40 ± 1.22	57.23 ± 1.29	53.92 ± 1.42*	57.70 ± 0.79
	Mean cell haemoglobin (pg)	19.92 ± 0.35	19.28 ± 0.29	18.52 ± 0.39*	19.23 ± 0.27
	Mean cell haemoglobin concentration (g/dl)	34.22 ± 0.22	33.80 ± 0.25	34.43 ± 0.36	33.83 ± 0.42
	Hematocrit (%)	41.08 ± 2.59	43.95 ± 2.04	46.02 ± 1.51	47.43 ± 1.09
	Platelet ($\times 10^9$/L)	578.33 ± 32.45	668.5 ± 53.73	786.33 ± 49.46**	805.33 ± 58.70*

Values are mean ± SD (n=6).
* $p < 0.05$.
** $p < 0.01$.
**** $p < 0.0001$ significantly different from control (One-way ANOVA followed by Dunnet's test).

Table 4
Serum biochemical parameters in rats after 28 and 90 days of daily administration of *Markhamia tomentosa* leaf extract.

Study	Parameter	Dose (mg/kg)			
		Control	40	200	1000
Sub-acute (28 days)	Alkaline phosphatase (U/L)	84.5 ± 3.02	87.5 ± 2.59	86.17 ± 2.23	86.00 ± 1.55
	Aspartate transaminase (U/L)	53.5 ± 5.01	58.00 ± 5.18	50.67 ± 5.05	57.00 ± 7.21
	Alanine transaminase (U/L)	18.0 ± 2.76	19.83 ± 2.40	19.83 ± 2.71	22.00 ± 3.29
	Albumin (mg/dL)	3.77 ± 0.33	4.07 ± 0.16	3.98 ± 0.15	3.90 ± 0.14
	Total bilirubin (mg/dL)	0.65 ± 0.10	0.57 ± 0.10	0.58 ± 0.15	0.60 ± 0.14
	Triglyceride (mg/dL)	38.00 ± 8.22	45.5 ± 11.76	54.00 ± 11.12*	36.33 ± 4.76
	Cholesterol (mg/dL)	114.33 ± 10.91	104.33 ± 26.56	107.83 ± 14.47	96.83 ± 6.59
	High-density lipoprotein (mg/dL)	27.5 ± 2.88	29.33 ± 3.39	30.67 ± 3.72	31.5 ± 3.02
	Total protein	7.7 ± 0.36	8.10 ± 0.28	8.25 ± 0.24*	8.23 ± 0.33*
Chronic (90 days)	Alkaline phosphatase (U/L)	76.83 ± 1.17	76.67 ± 1.21	76.67 ± 1.21	76.83 ± 0.98
	Aspartate transaminase (U/L)	47.33 ± 4.23	71.83 ± 7.44****	63.67 ± 9.31**	61.67 ± 6.68**
	Alanine transaminase (U/L)	14.50 ± 2.07	30.37 ± 3.39***	24.00 ± 4.34*	24.67 ± 9.00*
	Albumin (mg/dL)	4.45 ± 0.32	5.03 ± 0.33*	5.10 ± 0.23**	4.92 ± 0.36*
	Total bilirubin (mg/dL)	0.60 ± 0.14	0.67 ± 0.10	0.77 ± 0.16	0.75 ± 0.14
	Triglyceride (mg/dL)	105.83 ± 9.26	103.83 ± 13.44	97.5 ± 12.37	101.00 ± 10.14
	Cholesterol (mg/dL)	137.5 ± 37.22	130 ± 17.91	118.67 ± 12.03	121.67 ± 15.95
	High-density lipoprotein (mg/dL)	36.33 ± 11.84	32.17 ± 5.27	28.17 ± 3.19	32.67 ± 5.68
	Total Protein	8.62 ± 0.34	9.73 ± 0.49**	8.95 ± 0.63	8.72 ± 0.53

Values are mean ± SD (n=6).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
**** $p < 0.0001$ significantly different from control (One-way ANOVA followed by Dunnet's test).

rats. Also, a significant ($p < 0.05$) increase in TP at the dose of 40 mg/kg in the treated rats was observed. The chronic administration of the plant extract did not cause any significant changes in ALP, TBIL, TG, TCHO and HDL-c serum levels (Table 4).

3.5. Effect of plant extract on serum electrolytes, creatinine and urea in the 28 and 90 days studies

Markhamia tomentosa leaf extract did not produce any significant ($p > 0.05$) effect on the serum electrolytes, Cr and BUN after 28 day daily administration except for a significant ($p < 0.05$, 0.01) non-dose-dependent increase in Ca^{2+} concentration and decrease in URIC level in treated animals compared to the control (Table 5).

There was a significant ($p < 0.05$, 0.0001) non-dose dependent decrease in serum Na^+ and Ca^{2+} concentration at 200 mg/kg dose after chronic daily administration of the plant extract. Also, a significant ($p < 0.05$) decrease in serum Cr, BUN and URIC concentrations in treated compared to control group was observed (Table 5).

3.6. Effect of plant extract on histopathological assessment in the sub-acute and chronic studies

As shown in Figs. 1 and 2, microscopic examination of the tissues of the kidneys and liver showed unremarkable cellular

Table 5
Serum electrolytes, creatinine and urea levels in rats after 28 and 90 days of daily administration of *Markhamia tomentosa* leaf extract.

Study	Parameter	Dose (mg/kg)			
		Control	40	200	1000
Sub-acute (28 days)	Sodium (meq/L)	147.00 ± 2.61	148.67 ± 2.34	149.17 ± 4.12	143.17 ± 4.36
	Potassium (meq/L)	5.08 ± 0.22	5.70 ± 0.97	5.45 ± 0.71	4.58 ± 0.77
	Chloride (meq/L)	109.67 ± 3.14	110.00 ± 5.25	108.67 ± 4.46	103.83 ± 5.87
	Calcium (meq/L)	7.78 ± 0.15	7.56 ± 0.36	8.62 ± 0.42**	8.35 ± 0.33*
	Bicarbonate (meq/L)	26.33 ± 1.03	27.33 ± 2.07	25.67 ± 1.63	24.17 ± 1.60
	Creatinine (mg/dL)	1.08 ± 0.26	0.83 ± 0.08	0.98 ± 0.21	0.83 ± 0.15
	Urea (mg/dL)	28.33 ± 4.03	25.50 ± 3.02	26.83 ± 3.19	24.33 ± 4.32
	Uric acid (mg/dL)	3.73 ± 0.21	3.38 ± 0.41	2.68 ± 0.23**	2.98 ± 0.35*
Chronic (90 days)	Sodium (meq/L)	147.17 ± 5.42	145.33 ± 3.83	140.67 ± 2.50*	145.67 ± 3.61
	Potassium (meq/L)	5.15 ± 0.65	4.83 ± 0.74	4.13 ± 0.53	4.67 ± 0.58
	Chloride (meq/L)	106.67 ± 5.54	107.33 ± 4.89	101.00 ± 2.37	104.33 ± 4.97
	Calcium (meq/L)	10.12 ± 0.28	9.57 ± 0.45	8.75 ± 0.23****	9.88 ± 0.57
	Bicarbonate (meq/L)	25.17 ± 1.17	25.00 ± 1.41	23.50 ± 1.05	24.67 ± 1.21
	Creatinine (mg/dL)	0.98 ± 0.15	1.07 ± 0.17	0.8 ± 0.08	0.75 ± 0.11*
	Urea (mg/dL)	25.50 ± 4.37	29.50 ± 3.39	22.67 ± 2.50	19.83 ± 4.75*
	Uric acid (mg/dL)	6.75 ± 0.58	5.32 ± 1.38*	4.28 ± 0.46**	5.35 ± 0.65

Values are mean ± SD (n=6).
* $p < 0.05$.
** $p < 0.01$.
**** $p < 0.0001$ significantly different from control (One-way ANOVA followed by Dunnet's test).

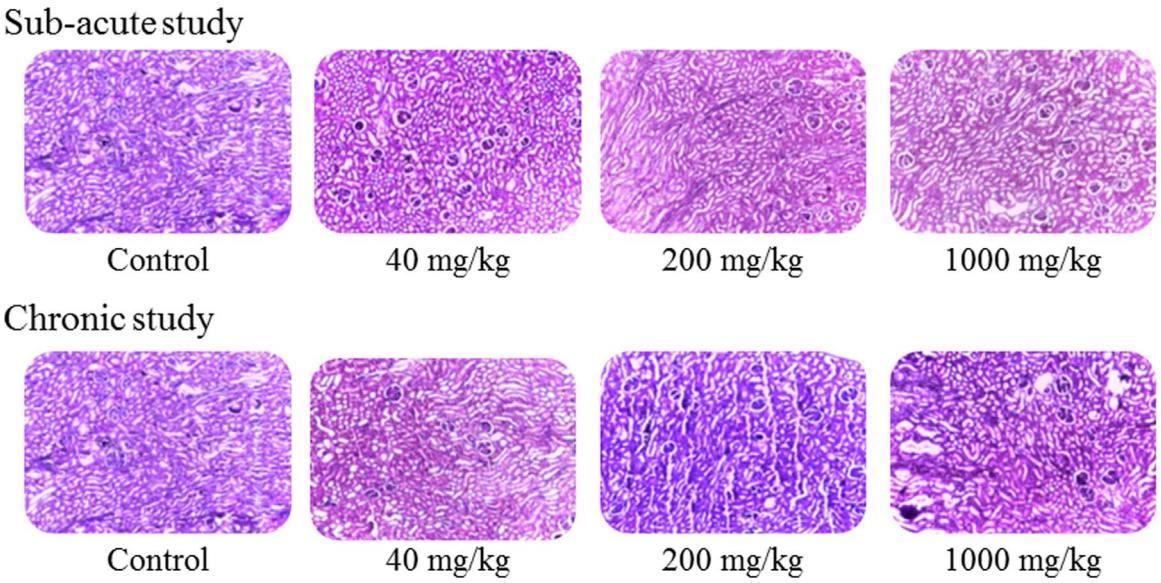

Fig. 1. Photomicrographs of sections of the kidneys in control rats and rats treated with doses of *Markhamia tomentosa* leaf extract daily for 28 and 90 days. No significant damage was observed in all treatment groups (× 100 magnification).

appearances with no alterations in the treatment and control groups of both sub-acute and chronic studies. Histopathological examinations of tissues from all the harvested organs indicated no treatment-related changes in the treated and control rats.

3. Discussion

Medicinal plants often contain highly active pharmacological compounds and this has been the basis for the treatment of various diseases (Wang et al., 2014). Characterisation of the chemical constituents of *Markhamia tomentosa* leaf extract by electrospray ionisation mass spectrometry (LC-MS) identified phenolic, terpenoid and iridoid compounds (Sofidiya et al., 2014). Although this plant contains bioactive compounds that have potential to cause beneficial and /or detrimental effects, a thorough toxicity study is important to ascertain its safety and efficacy.

The primary goal of evaluating the safety of any herbal drug is to identify the nature and significance of adverse effects as well as to determine the exposure level where the effect is observed. Some of the risks that may be associated with the use of herbs can be revealed through toxicity testing (Obidike and Salawu, 2013).

According to Sowemimo et al. (2013), mice treated with 5 g/kg dose of the leaf extract of *Markhamia tomentosa* showed no mortality or visible side effect in the acute toxicity study. This indicated that the plant extract is relatively safe as classified by the Organisation for Economic Cooperation and Development (OECD) Guidance Document for Acute Oral Toxicity Study (OECD, 2001; Tarkang et al., 2012). Acute toxicity data are of limited clinical application due to the fact that cumulative toxic effects could occur even at very low doses (Abotsi et al., 2011). Hence, in this present study, the sub-acute and chronic toxicity profile of

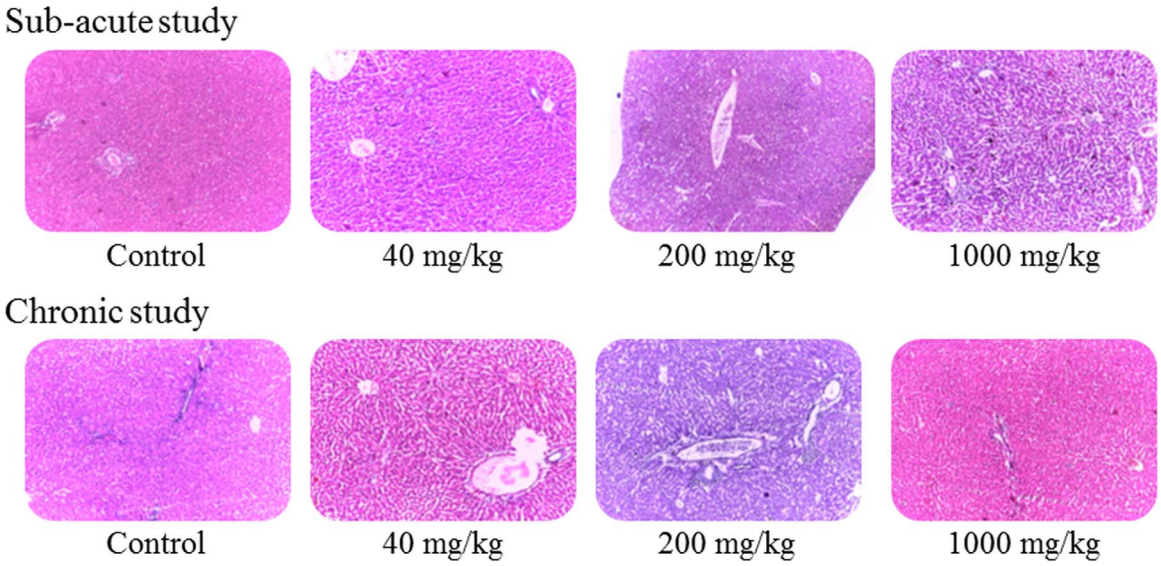

Fig. 2. Photomicrographs of sections of the liver in control rats and rats treated with doses of *Markhamia tomentosa* leaf extract daily for 28 and 90 days. No significant damage was observed in all treatment groups (× 100 magnification).

Markhamia tomentosa leaf extract were evaluated in rats using measurement of body and organ weights, haematological, biochemical and histopathological parameters.

Changes in body weight are sensitive indices of adverse effects of drugs and chemicals (Santos et al., 2009; Wang et al., 2014). Daily oral treatment of *Markhamia tomentosa* over the period of 28 and 90 days studies showed no significant changes in body weight gain pattern in the treated rats compared to the control rats. These results suggested that there was no effect on normal growth of rats at the administration of the chronic oral doses of *Markhamia tomentosa* leaf extract.

Analysis of blood parameters can be used to determine the extent of adverse effect of foreign compounds including plant extracts (Agbaje et al., 2009). Changes in the hematopoietic system have a higher predictive value for human toxicity when data are translated from animal studies (Olson et al., 2000). The haematology results obtained from this study showed significant increase in WBC, PLT, RBC and MCV and a significant decrease in RBC, MCV, MCH and MCHC parameters compared to their respective controls after 28 and 90 days oral treatment with *Markhamia tomentosa* leaf extract. No significant changes were observed in other parameters.

Hematopoiesis is the process of blood cell formation and maturation in the bone marrow. All blood cells are derived from the pluripotent hematopoietic stem cell, an immature cell capable of becoming a leucocyte, an erythrocyte or a thrombocyte (Guyton and Hall, 2006). After 28 days oral administration of *Markhamia tomentosa* leaf extract to the animals, the significant decrease in RBC parameter at the highest dose indicated that the plant extract adversely affected the erythropoietic system. The observed significant increase in MCV and significant decrease in MCHC parameters at the lowest and medium doses could suggest an increase in the average size of RBC and decrease in haemoglobin weight per RBC (Agbaje et al., 2009).

By the 90th day of repeated oral treatment, there was a significant dose dependent increase in WBC and PLT and non-dose-dependent increase in RBC parameters. This implied that the plant extract was capable of stimulating the hematopoietic system leading to the production of WBC (leukopoiesis), PLT (thrombopoiesis) and RBC (erythropoiesis). The significant ($p < 0.0001$) elevation of WBC may indicate the impact of *Markhamia tomentosa* leaf extract in boosting the immune system of the treated rats. Also, the plant extract being able to significantly decrease MCV parameter following chronic oral administration could make it efficient in the treatment of macrocytic anaemia (Kasper et al., 2005; Adebayo et al., 2015). The significant decrease in MCH parameter is justified by the significant decrease in MCV as they have been reported to be mirror images (Davidson and Hamilton, 1978).

The clinical biochemistry analyses were carried out to evaluate possible alterations in the hepatic, renal and lipid profiles of treated compared to control rats, in order to detect possible pathological changes and nature of disease. *Markhamia tomentosa* extract showed no significant changes in all the biochemical parameters in the 28 day treatment period except for a significant increase in TP, TG and Ca^{2+} parameters at the medium and highest doses. Measurement of TP and ALB parameters can represent nutritional status and may be used to screen for and assist in the diagnosis of liver and kidney diseases (Thierry et al., 2011; Patrick-Iwuanyanwu et al., 2012). Increase in these parameters is reported to have hepato-protective effect (Oyagbemi et al., 2008; Mbaka et al., 2014). The significant increase in the TP parameter and non-significant increase in ALB parameter observed in this study may suggest potential hepatoprotective effect of *Markhamia* leaf extract. Determination of serum electrolytes, Cr, BUN and URIC parameters are important markers of kidney function and elevations in the levels of these parameters are indicative of kidney injury (Woodman, 1996; Arneson and Brickell, 2007; Akindele et al., 2014). The extract at the medium and highest doses caused significant decrease in URIC level with no observable significant changes in other serum electrolytes, Cr and BUN parameters in the treated compared to control rats. These results indicated that the plant extract has no toxic effect on the kidneys.

Changes in the concentration of major lipids such as TCHO, HDL-c and TG can give necessary information on lipid metabolism and predisposition of the heart to atherosclerosis and other associated coronary heart diseases (Yakubu et al., 2008; Wang et al., 2014). The result obtained from this treatment period showed non-significant decrease in TCHO and HDL-c parameters but a significant increase in TG parameter of the treated rats at the medium dose of the extract. The observed decrease in TCHO is indicative of the presence of hypolipidaemic agent in the plant extract, suggesting potential beneficial effect against cardiovascular risk factor (Zhou et al., 2006).

Repeated oral treatment with *Markhamia tomentosa* leaf extract

for 90 days was associated with significant, non-dose-dependent increase in the hepatic profile (ALT, AST, ALB and TP) parameters in treated rats compared to control rats. There was a significant reduction in the renal profile (Na^+, Ca^{2+}, Cr, BUN and URIC) parameters while the lipid function profile, serum TCHO, HDL-c and TG levels, was not significantly altered compared to the control. The liver and kidneys are the two major organs that play important roles in detoxification process. ALT, AST and ALP are the most common parameters used to assess the function of the liver (Tolman and Rej, 1999). ALT is the most sensitive serum marker enzyme for liver damage. It is produced within the cells of the liver and provides quantitative assessment of the degree of damage sustained by the liver (Al-Mamary et al., 2002). Unlike ALT, AST is not a highly specific marker for liver damage as it can be found in other tissues like the heart, brain, muscles and kidney (Adeyemi et al., 2010). ALB is a major protein synthesised in the liver and also an important biomarker for liver diseases (Adebayo et al., 2015). The significant increase in ALT and AST parameters observed in this study might be linked to an unknown factor as the plant extract caused profound increase in the levels of ALB and TP indicative of hepato-protection (Oyagbemi et al., 2008; Mbaka et al., 2014). In the event of acute or chronic renal toxicity, serum electrolytes, Cr, BUN and URIC parameters are usually markedly increased in treated compared to control animals (Newman and Price, 1999; Arsad et al., 2013). The results obtained from this study showed significant decrease in serum Na^+, Ca^{2+}, Cr, BUN and URIC levels in the treated compared to control rats which indicated that *Markhamia tomentosa* leaf extract has no toxic effects on the kidneys. The insignificant changes in the lipid function profile, serum TCHO, HDL-c and TG levels suggested that the activity of lipid metabolism was maintained within the normal range due to relatively non-toxic effect of the plant extract.

Generally, reduction in internal weight of an organ is an indication of toxicity following exposure to toxic substances (Raza et al., 2002; Adeyemi et al., 2010). In respect to the harvested vital organs, no significant changes in weight relative to the control were observed in all organs in the 28 day treatment period except for a significant increase in weight of the lungs at the medium and highest doses. More so, the hepatic tissue histology did not show any pathological changes. The weight of the lung is less important in toxicity studies due to its low frequency of finding weight changes that correlate with toxicity and it is less sensitive to predict toxicity compared to histopathology (Michael et al., 2007; Amna et al., 2013). Therefore, since the weight of the target organs for toxicity which are the liver and kidneys were not significantly altered, it could be claimed that the plant extract does not produce any toxic effect on the harvested organs of the treated compared to control rats in the sub-acute study.

A significant non-dose-dependent increase in weights of liver and testes and a significant non-dose-dependent decrease in the weight of the brain were observed in the 90 day treatment period. No significant changes were observed with the weights of the heart, lungs, kidneys and spleen in this treatment period. In addition, no treatment-related histological alterations were observed in any of the microscopic slides of tissues of the harvested organs examined. Kidney and liver weights have been considered useful in toxicity studies due to its sensitivity to predict effect of toxicity and correlates well with histopathological changes (Amna et al., 2013). *Markahmia tomentosa* did not cause any significant histopathological changes in the liver of the treated rats. The plant extract also caused significant increase in the biochemical ALB and TP suggestive of hepato-protection. The observed increase in weight of the liver in the chronic study may however be attributed to unknown cause. Lack of histopathological changes and insignificant alterations of the weights of the kidneys and heart correlated with the results of the renal and lipid profile parameters suggesting that the plant extract does not exert toxic effect on the functions of the kidneys and the heart in both sub-acute and chronic toxicological studies. However, due to the significant reduction in the brain weight, further study is needed to ascertain the toxic effect of the plant extract on the brain.

4. Conclusion

The findings from this investigation provide valuable data on the sub-acute and chronic oral toxicological profile of *Markhamia tomentosa* leaf extract. The plant has been reported to be non-toxic on acute exposure (Sowemimo et al., 2013), and has shown from the results obtained from this study to be generally safe on sub-acute and chronic administration. Prolonged administration revealed effects which suggest that the extract exhibited no toxic effects with regard to the body and relative organ weights, haematological, biochemical and histopathological parameters of rats. The results observed at the treatment doses indicated potential for boosting components of the immune system and protecting the cardiovascular, liver and kidney systems. Furthermore, the indices pertaining to hepatic and renal functions showed no abnormalities. These observations suggest that leaf extract of *Markhamia tomentosa* is not likely to produce any toxicological effects and is safe for medicinal use.

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