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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