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Chronic LD50 vs safest dose for the methanolic extract of curry leaves (*Murraya koenigii*) cultivated in Malaysia

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ABSTRACT

Curry leaf viz. *Murraya koenigii*l eaves (MKL) is an ancient Ayurvedic medicinal plant that has recently been described as possessing robust anti-inflammatory and antioxidant activities. The current study was conducted to determine the long-term LD50 of the methanolic extract of MKL during daily oral administration. Five groups of Sprague Dawley rats were recruited into the study. Each group comprised of six rats including the control group (1). The oral MKL doses used for groups (2) to (5) were 50, 100, 200 and 400 mg/kg/day, respectively for a duration of ten weeks. The daily measured toxicity parameters were food and water consumption, body weight, general activity in forced swimming test and the cumulative mortalities. Group (5) showed 100% mortality within the first month of the study. Group (4) showed 50% mortality with signs of toxicity for the other 3 animals. Group (3) showed no mortalities but signs of toxicity for one animal were observed. No mortalities or toxicity signs were observed for any of group (2) animals. It can be concluded that the chronic LD50 for Malaysian cultivated MKL is 200 mg/kg/day, and the safest dose of MKL methanolic extract that can be implemented for long-term studies should not exceed 50 mg/kg/day.

INTRODUCTION

Belonging to the plant family "rutaceae", *Murraya koenigii* leaves (MKL) is a traditional known spice and flavoring food additive. In Ayurvedic medicine the leaves were customarily used for the treatment of various GI tract disorders ranging from nausea to diarrhea (Ghani, 2003). Because MKL has recently shown robust antioxidant, anti-inflammatory and antinociceptive activities (Patil *et al.*, 2012; Tachibana *et al.*, 2001), it started to gain more consideration to be applied for therapeutic objectives of longer durations. The characteristic nature of MKL of having a wide range of diversity in its chemical compounds composition among plants cultivated in different region of the world (Chowdhury *et al.*, 2008; Rao *et al.*, 2011) has led to two dynamic outcomes. The first is that MKL extract taken out from leaves implanted in one area of the world can have therapeutic uses different form the same plant extract that was cultivated in other environmental conditions. The second is that the toxicity profile of the plant extract may also differ according to the nature and the concentration of the chemical constituents within the extract itself. Therefore, it was of paramount importance to perform a locally independent study to find out the chronic LD50 for MKL methanolic extract that was exclusively cultivated in Malaysia. This could be the cornerstone for future long-term researches on Malaysian cultivated MKL extract.

MATERIALS AND METHODS

Animals

30 adult male Sprague Dawley rats were randomly and equally divided into 5 groups. Except for the control group (1) which only received the vehicle, each group received a different dose of MKL extract *per os* via gavage cannula for a period of 10 weeks. The daily oral doses for groups (2) to (5) were 50, 100, 200 and 400 mg/kg, respectively.

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Animals were housed as singles per cage and had free access to water and food pellets of defined weight (1g) throughout the study with equal daily exposure to light and darkness. The framework principles of the 'Office International des Epizooties' (OIE) on ethical use of animals were carefully followed. The toxicity parameters recorded on daily basis were the animal's body weight, volume of water, number of food pellets consumed and the general activity of each animal as well as its exploratory behaviors.

MKL extract preparation

Fresh curry leaves (10 kg) were harvested from Sungai Pandan Forest, Kuantan. MKL were identified and authenticated by a taxonomist in the Faculty of Pharmacy, IIUM, Kuantan campus. Voucher specimen was deposited at Faculty's herbarium. Leaves were left to dry before they were ground to powder and dissolved in methanol under magnetic stirrer for 24 hours. The pure extract was separated on the next day using rotary Evaporator apparatus. The total yield of MKL extract was 198 g.

Statistical analysis

General linear model (GLM) repeated measure ANOVA was implemented using SPSS software version 17.0 to analyze inter group differences at each time interval. Results were expressed as mean (SD). ***P*** values lower than 0.05 were regarded statistically significant.

RESULTS AND DISCUSSION

The significance of performing chronic toxicity study for MKL extract is the recently accumulating body of evidences reporting the potential therapeutic values of this extract in diseases that have chronic courses of illnesses, especially its promising antiamnesic (Vasudevan & Parle, 2009) and antidiabetic properties (Dineshkumar *et al.,* 2011). Acute toxicity tests demonstrated that the LD50 of MKL extract given as a single dose can reach up to 2500 mg/kg (Darvekar *et al.,* 2011). However, in other studies toxic side effects appeared at much lower doses (Adebajo *et al.,* 2006). These toxic effects were manifested as hepatic and renal dysfunctions. Yet, the duration of treatment for the applied doses in these studies were not sufficient to be regarded as chronic. Moreover, reports of quantitative and qualitative chemical diversity in the essential oils of MKL ingredients from different origins (Rao *et al.,* 2011) necessitated the implementation of long-term toxicity study to determine the chronic LD50 of Malaysian cultivated MKL in particular.

Figure 1 illustrates the variations in toxicity parameters among all groups after each 10 day time interval. The 100% mortality of group (5) after the first month and the significant toxicity signs expressed by group (4) especially for its deteriorating water and food consumption during the last month of the study represent a solid evidence for the inappropriate use of such doses for long-term treatment with MKL extract. This is especially true if we notice that half of the animals in group (4) died after day 60. Although the overall results of the toxicity parameters of group (3) which received 100 mg/kg/day were closely resembling those of group (2) which received 50 mg/kg/day, the significantly reduced animals' activity and food consumption during the last 20 days may indicate that 100 mg/kg/day is still above the margin of safety to be used for long-term studies. Interestingly, weight reduction was noticed even with the lowest dose (50 mg/kg/day) of MKL treatment which supports previous reports which stated that MKL can effectively lead to weight reduction owing to its high fibers contents (Khanum *et al.,* 2000).

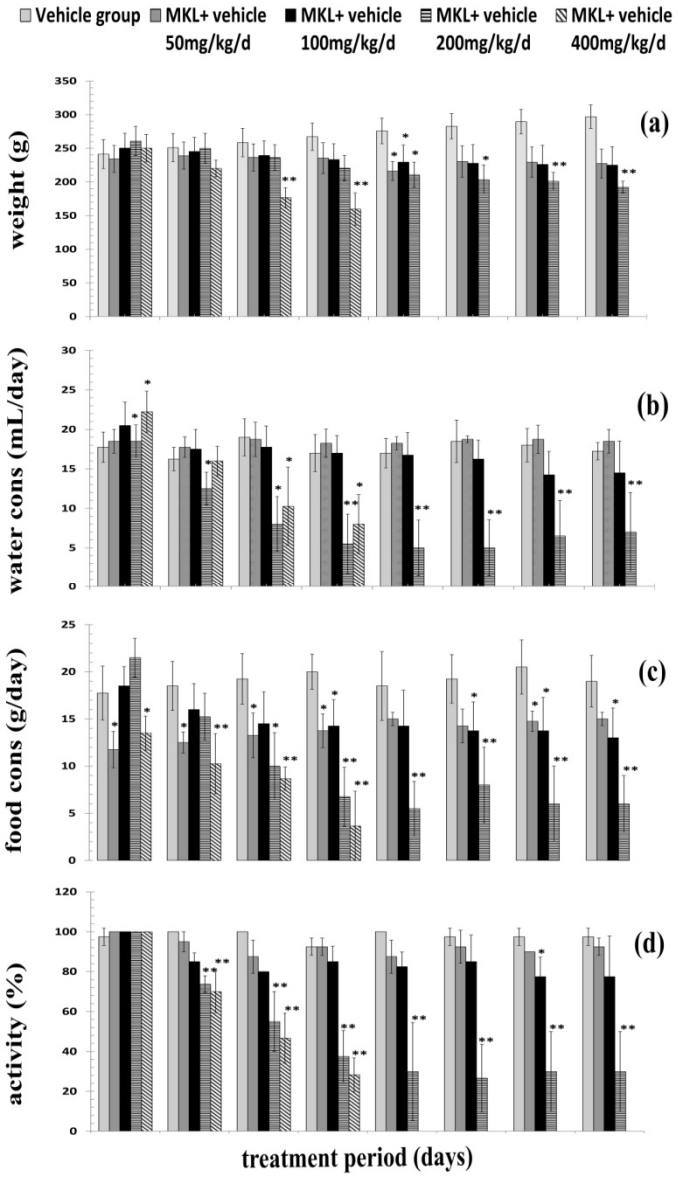

Fig. 1: Toxicity parameters used to evaluate MKL long-term toxic side effects at 10 days intervals. * ***P***<0.05, ** ***P***<0.01 vs control group.

Apoptotic cell death is thought to be one of the key mechanisms of toxicity imposed by three major carbazole alkaloids of MKL (mahanine, pyrayafoline-D and murrafoline-I)

through interference with mitochondrial function intracellularly (Ito *et al.,* 2006). The (SEM) standard error of the mean for the LD50 was estimated according to the method previously described by (Ghosh, 1984) and was found to be 33.82.

As a conclusion the chronic LD50 for Malaysian cultivated MKL methanolic extract was 200 ± (33.82) mg/kg/day. The safest dose which didn't show any long-term toxic side effect was 50 mg/kg/day. Further biochemical and vital organ histopathological analyses are required to ensure safety of the abovementioned dose in more comprehensive studies.

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