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Toxicological evaluation of dried kacangma (*Leonurus sibiricus*) in rats: II. Haematology and histopathology

[Penilaian ketoksikan kacangma kering (*Leonurus sibiricus*) pada tikus: II. Hematologi dan histopatologi]

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Key words: *Leonurus sibiricus*, toxicity evaluation, haematology, histopathology, *Sprague Dawley* rats

Absract

Kacangma (*Leonurus sibiricus* L.) is a popular traditional herb among the people of Sarawak as herbal medicine and culinary ingredient. The toxicity of kacangma herb on male and female *Sprague Dawley* rats was evaluated in the sub-chronic studies. Effect of kacangma intake at 0.5, 5.0 and 25.0 g/kg body weight was evaluated for 90 days with haematological and histopathological analysis. The haematology study revealed statistically significant mild anaemia in rats from the medium and high dose groups as indicated by decreases in haemoglobin, red blood cell count and packed cell volume (haematocrit value). Administration of kacangma herb at medium and high dose was also found to cause adverse effects in histopathological structure of the liver and kidney of both male and female rats. However, low dose group showed no significant differences compared to the control. Therefore, it is considered safe and less chance of developing toxicity if the herb is consumed at the dose of 0.5 g/kg body weight as observed throughout the 90-day period of sub-chronic study.

Introduction

Kacangma (*Leonurus sibiricus* L.) is a popular traditional herb which has been consumed for years by the people of Sarawak as traditional medicinal and culinary herb. It is believed to be effective in improving blood circulation, treatment of cardiovascular diseases and gynecological disorders in women (Teo and Chua 2001). The role of kacangma as a local herb with potential economic value has been recognized (MOA 1995, Paulus and Lau 2004). Consequently, efforts are made to reevaluate its values; as well as to increase its utilization by developing into various specialty herbal products with commercial significance.

Dried kacangma herb has been consumed for many generations and is generally considered safe and harmless mainly due to its natural origin (*Plate 1*). To date, the toxicity of kacangma herb has not been extensively substantiated with scientific and clinical research. There is not much information on its side effects that may compromise the safe use of this herb.

Toxicological studies are necessary to indicate the safe level of exposure of

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Plate 1. Dried kacangma herbs

Plate 2. Kacangma herb (***Leonurus sibiricus*** L.) *at 2-month maturity*

herbs used as folk medicinal and culinary ingredients. There have been cases of unreported adverse reactions of herbal preparations especially in the developing countries (Dioka et al. 2002). According to Ur-Rahman and Zaman (1989) and Guil et al. (1997), a number of anti-nutritional and highly toxic compounds have been isolated from plants. Furthermore, it has been reported that prolonged period of consumption of traditional herbal preparations such as ginkgo, St. John Wort, ginseng, echinaceae and ephedra is associated with toxic effects on kidney, liver, lung, cardiovascular and central nervous systems. Even common culinary herbs such as onion and garlic may cause toxic effects on liver and lung if consumed at high dosage (Alnaqeeb et al. 1996; Thomson et al. 1998; Kadiri et al. 1999; Haller and Benowitz 2000; Ernst 2002).

Previous study on 90-day sub-chronic study of dried kacangma in *Sprague Dawley* rats has been carried out on blood chemistry, body and organ weight changes (Chua et al. 2006). This study was conducted as the continuation to further evaluate the effect of dried kacangma with focus on haematology and histopathology analysis.

Materials and methods

Preparation of formulated kacangma pellet

Formulated kacangma pellet was prepared by mixing the dried kacangma powder at certain fixed ratios with a commercial rat pellet [Gold Coin Feedmills (M) Sdn. Bhd.]. Dried kacangma leaves were processed by drying the freshly harvested 2-month-old kacangma herb (*Plate 2*) at 45 °C in a force-air oven (Chua et al. 2006). Dried kacangma was powdered and added to the rat pellet and water was used to bind the mixture. The homogenized paste-like mixture was then reformed into pellets of 0.5 g average weight before drying in the oven at 45 °C until final moisture content was below 6% (w/w). The formulated kacangma pellets were then stored at chilled temperature in airtight containers to prevent mould growth. Similar feed preparation method was used by Suhaila et al. (2001) in pegaga (*Centella asiatica*) efficacy test on rats.

According to WHO (1993), at least three different dose levels should be used in long-term toxicity test. One dose level (low dose) should not cause toxic changes and another one dose level (high dose) that produces overt toxic effects should be included. The low dose 0.5 g/kg body weight in this study was formulated based on the normal dose use of kacangma herb in human consumption, as well as the homeopaths' recommended level of active constituents (alkaloid leonurine and stachydrine) in most *Leonurus* species (Yeung et al. 1977; Bradley 1992). The high dose was calculated as 50 times of low dose. Since the body weight of rat is much lower as compared to human, the dose level of kacangma has been calculated based on the ratio average body weight of human vs. rats

i.e. 0.5 (low dose), 5.0 (medium dose) and 25.0 (high dose) g/kg of body weight.

Experimental animals

A total of 40 *Sprague Dawley* rats (20 males and 20 females) each weighing average 180–205 g were acclimatized for two weeks before starting the study. According to the guidelines for toxicity investigation of herbal products using rats (rodents) as experimental animals, at least five animals per group of a certain sex should be used for long-term (sub-chronic and chronic) toxicity test to be statistically validated (WHO 1993).

The rats were housed individually in elevated stainless steel cages and kept at 25 °C with a 12-h light/dark cycle. The rats were provided with formulated kacangma pellets and water *ad libitum* (free access for 24 h). Animal identification was via cages, with colour-coded identification card, indicating the animal and treatment groups.

At the end of the experiment, all rats were fasted overnight for at least 15 h and euthanasied with chloroform. The gross examination included the external surfaces, all orifices, all visceral organs, the cranial, thoracic, abdominal and pelvic cavities.

Sub-chronic toxicity

In sub-chronic toxicity study, a 90-day repeat dose oral study was conducted to evaluate the potential toxicity of kacangma herb. The rats were equally distributed into four groups (10 rats per group, 5 males and 5 females). Group 1, 2 and 3 were fed formulated kacangma pellets each day with a dried kacangma content of 0.5 (low dose group), 5.0 (medium dose group) and 25.0 (high dose group) g/kg of body weight, respectively for 90 days. Group 4 served as a control and was fed only commercial rat pellet. Individual food consumption and water intake were recorded weekly.

Haematology studies

After 90 days of feedings, all rats were fasted overnight prior to blood collection. Approximately 1.0 ml of blood was collected from each rat via intracardiac puncture. The blood was transferred into tube containing ethylene diamine tetra-acetic acid (EDTA) as anti-coagulant solution. Immediately soon after, the blood was gently mixed by inverting the tube at least 6–10 times to avoid clotting. The fresh whole blood was determined for haematology values using Cell-Dyn Counter (Abbottes).

Parameters studied are haemoglobin (HB), white blood cell (WBC), red blood cell (RBC), packed cell volume/haematocrit value (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). MCHC was calculated by dividing HB by PCV.

Histopathological examination

The histopathological examination was conducted according to method described by Luna (1986) and Dioka et al. (2002). After taking blood samples for the haematology studies, all rats were killed by cervical dislocation under chloroform anaesthesia and necropsied. The five main organs (kidney, liver, lung, heart and spleen) were quickly excised and immediately weighed after being rinsed in 0.9% cold saline to remove excess blood. These organs were examined for evidence of gross lesions. Representative tissue samples of each organ were fixed in 10% neutral buffered formalin for routine histopathology analysis. Tissue sections were stained by haematoxylin and eosin method (H&E) and were examined and photographed by a histopathologist using a light microscope. Histopathological examination was carried out at Histopathology Laboratory, Faculty of Veterinary, UPM.

Statistical analysis

The significant differences between the control and kacangma herb-treated rats were determined using ANOVA at 5% level (p <0.05) followed by DMRT. All values are expressed as group mean ± standard error of mean.

Results and discussion

Haematological values

The effect of kacangma feed on haematological values in male and female rats are shown in *Table 1*. Haematology study has been recognized as an easy and readily screen for haematotoxicity. For instance in human, the study is one of the series of tests that normally carried out in pathology laboratory to determine disease or toxic effect (Halimah 2001)

Haemoglobin (HB) is an iron-containing protein that is found in red blood cells which primary function is to transport oxygen from the lung to the body. A decrease in HB normally occurred in anaemia, haemorrhage or severe bleeding cases. The white blood cell volume (WBC) is studied for disorders either leucopoenia (decrease in number of cells) or leukocytosis (increase in number of cells), whereas red blood cell volume is studied for disorders either anaemia (decrease in number of cells) or polycythaemia (increase in number of cells). Packed cell volume (PCV) is a measure in percentage of the relative volume of plasma, the total red blood cell mass and the concentration of red blood cells. It is also referred as haematocrit value. A decrease in PCV will indicate anaemia or leukaemia (Vaughn 1999).

The haematology study revealed statistically significant mild anaemia in the medium and high dose male and female rats. This was indicated by significant decreases in haemoglobin, red blood cell count and packed cell volume for rats in these two groups. The nature of anaemia could not be determined in this study but may due to the therapeutic effects of kacangma herb as an anti-hypertensive agent with potential in reducing blood viscosity and activating blood circulation. Chen and Kwan (2001) reported that one of the therapeutic use of *Leonurus* herb was based on its improvement of circulation by removal of blood stagnation.

No significant differences were detected in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) between all groups of rats.

Histopathological findings

The results of histopathological examination of five main organs (kidney, liver, lung, heart and spleen) in the different treatment groups of rats are shown in *Table 2*. No abnormalities were observed in histological structure of heart and spleen for all rats. This indicated that there were no adverse effects in the heart and spleen of all rats, including rats that were administered with kacangma herb at high dose level.

Histopathological changes in the lung was found to occur randomly in some male and female rats in control and treated groups. This was consistent to antigenic stimulation due to viral or bacterial infections and is of doubtful toxicological significance since it occurred in both the control and treated rats.

Long-term intake of kacangma herb at medium and high dose was found to cause a degree of histopathological changes in liver and kidney of both male and female rats. These changes were mild to moderate in the medium dose level and severe in the high dose level. However, no abnormalities of liver and kidney were observed in control and low dose groups. Administration of kacangma herb was found to affect the liver and kidney at medium and high dose levels.

Changes of histopathological structure in liver of rats at medium and high dose groups included billiary hyperplasia and degenerative of hepatocyte cells (*Plate 3*). The liver is the principal organ that engaged a central role in major functions of organism due to its interposition between the digestive tract and the rest of the body. It receives a big amount of nutrients as well as toxic chemical residues into the body through the digestive tract and portal vein. The capability of a chemical to cause liver damage often results from the interaction of a series of complex cellular processes that

Table 1. Effects of kacangma feed on hematological values in male and female rats (n = 5)

	Control		Low dose (0.5 g/kg)		Medium dose (5.0 g/kg)		High dose (25.0 g/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Haemoglobin (HB) (g/dl)	13.40 ± 0.25a	13.36 ± 0.24a	11.53 ± 0.22b	11.45 ± 0.14b	11.15 ± 0.87b	10.44 ± 0.22b	11.66 ± 0.70b	10.23 ± 0.62b
White blood cell (WBC) (mm^3)	5350 ± 426.91a	6100 ± 63.25a	6020 ± 287.05a	5920 ± 477.91a	4480 ± 159.37b	4280 ± 159.37b	4020 ± 73.48b	4020 ± 66.33b
Red blood cell (RBC) (1012/litre)	6.42 ± 0.49a	6.30 ± 0.63a	6.78 ± 0.15a	6.66 ± 0.20a	5.30 ± 0.18b	4.94 ± 0.98b	5.28 ± 0.37b	5.18 ± 0.34b
Packed cell volume (hematocrit value) (PCV) (%)	33.00 ± 0.89a	33.00 ± 0.32a	34.60 ± 0.81a	35.00 ± 0.55a	27.60 ± 1.00b	25.60 ± 0.51bc	27.60 ± 1.25b	24.80 ± 0.37c
Mean cell volume (MCV) (fl)	50.40 ± 0.68a	52.40 ± 0.24a	50.60 ± 0.24a	53.60 ± 0.24a	52.00 ± 0.32a	52.40 ± 0.24a	50.20 ± 1.02a	49.60 ± 0.93a
Mean cell haemoglobin (MCH) (pg)	20.40 ± 0.51a	19.60 ± 0.24a	19.80 ± 0.20a	20.20 ± 0.37a	19.00 ± 0.32a	18.60 ± 0.24a	19.80 ± 1.85a	18.40 ± 0.24a
Mean cell haemoglobin concentration (MCHC) (%)	38.00 ± 0.32a	37.60 ± 0.24a	39.20 ± 0.20a	37.40 ± 0.51a	36.60 ± 0.60a	36.60 ± 0.51a	38.40 ± 2.20a	36.60 ± 0.24a

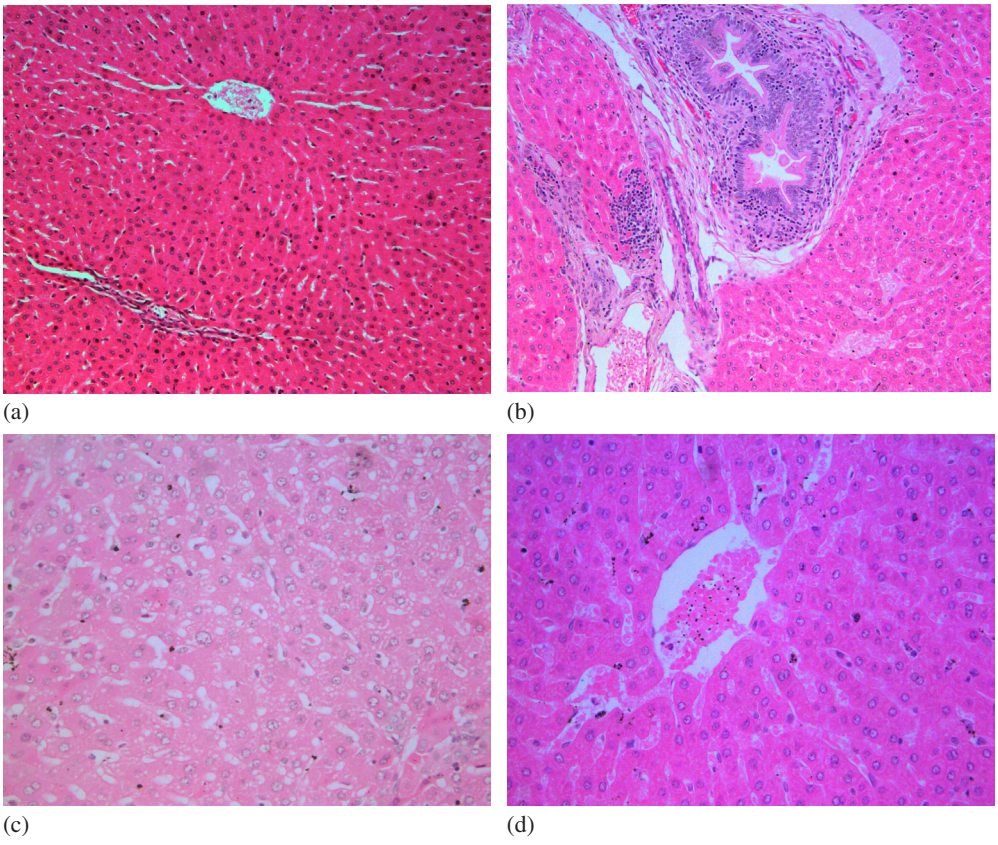
Mean values in the same row with the same latter are not significantly different ($p > 0.05$)

Table 2. Histopathological examination of main organs in male and female rats (n = 5)

		Liver	Kidney	Lung
Control	Male	All showed NA	All showed NA	• 3 showed NA • 2 showed severe pulmonary congestion and brochopneuminia
	Female	All showed NA	All showed NA	• 2 showed NA • 3 showed severe pulmonary congestion and brochopneuminia
Low dose (0.5 g/kg)	Male	All showed NA	All showed NA	• 3 showed NA • 1 showed moderate pulmonary congestion • 1 showed thickening of alveolar wall and lymphoid aggregation
	Female	All showed NA	All showed NA	• 4 showed NA • 1 showed lymphoid aggregation at bronchiol
Medium dose (5.0 g/kg)	Male	• 1 showed NA • 4 showed mild degeneration biliary hyperplasia and megalocytosis lymphocytic infiltration, with a lot of kupfer cells in the sinosoid of the liver	All showed renal nephrosis	• 2 showed lymphoid aggregation at bronchiol • 2 showed severe pulmonary congestion and brochopneuminia • 1 showed severe pulmonary congestion and oedema
	Female	• 2 showed NA • 3 showed mild degeneration biliary hyperplasia and megalocytosis lymphocytic infiltration, with a lot of kupfer cells in the sinosoid of the liver	All showed renal nephrosis	• 2 showed NA • 2 showed lymphoid aggregation at bronchiol • 1 showed severe pulmonary congestion and brochopneuminia
High dose (25.0 g/kg)	Male	• 2 showed severe degeneration biliary hyperplasia with bridging and degenerative of hepatocyte cells and double nuclueous • 3 showed severe degeneration biliary hyperplasia with bridging and degenerative of hepatocyte cells	All showed renal nephrosis	• 3 showed NA • 1 showed severe pulmonary congestion and brochopneuminia
	Female	• 4 showed severe degeneration biliary hyperplasia with bridging and degenerative of hepatocyte cells • 1 showed mild degeneration biliary hyperplasia and megalocytosis lymphocytic infiltration, with a lot of kupfercells in the sinosoid of the liver	All showed renal nephrosis	• 2 showed NA • 2 showed lymphoid aggregation at bronchiol • 1 showed severe pulmonary congestion and brochopneuminia

NA = No abnormalities in histopathological structure

Heart and spleen showed no abnormalities in histopathological structure



(a) (b)

(c) (d)

Plate 3. Representative photographs (40x) of liver sections from the control and high dose groups stained with haematoxylin-eosin: (a) A control rat showing normal liver histopathological structure; (b) A medium-dose treated rat showing liver with mild degeneration billiary hyperplasia; (c) A medium-dose treated rat showing liver with mild degeneration billiary hyperplasia and megalocytosis lymphocytic infiltration, with a lot of kupfer cells in the sinosoid of the liver; (d) A high-dose treated rat showing liver with severe degeneration billiary hyperplasia with bridging and degenerative of hepatocyte cells

are involved in the intake, biotranformation and elimination of these potentially toxic compounds (Guillouzo 1998).

All rats in medium and high dose groups of kacangma herb showed renal nephrosis. Nevertheless, no abnormalities of kidney were observed in control and low dose groups. This indicated that the administration of kacangma herb at medium and high dose levels will cause adverse effects on the kidney.

Conclusion

Haematology study reveals statistically significant mild anaemia in rats from the medium and high dose groups as indicated by decreases in haemoglobin, red blood cell count and packed cell volume (haematocrit value). Administration of kacangma herb at medium and high dose was also found to cause a degree of histopathologic changes in liver and kidney of both male and female rats. Low dose group (0.5 g/kg body weight) showed no significant differences to the control group, hence were considered safe as observed throughout the 90-day period of sub-chronic study.

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Abstrak

Kacangma (*Leonurus sibiricus* L.) ialah herba tradisional yang popular di kalangan penduduk Sarawak sebagai herba ubatan dan ramuan di dalam masakan. Ketoksikan kacangma kering terhadap tikus *Sprague Dawley* jantan dan betina telah dinilai melalui kajian ketoksikan sub-kronik. Kesan pengambilan kacangma kering pada aras 0.5, 5.0 dan 25.0 g/kg berat badan terhadap tikus ditentukan selama 90 hari melalui kajian hematologi dan histopatologi. Kajian hematologi memperlihatkan keadaan anemia yang sederhana secara bererti pada tikus dari kumpulan dos sederhana dan tinggi sejajar dengan penurunan nilai hemoglobin, sel darah merah dan isi padu sel padat (nilai hematokrit). Pengambilan herba kacangma pada dos sederhana dan tinggi turut menyebabkan perubahan yang bererti pada histopatologi hati dan ginjal tikus jantan dan betina. Walau bagaimanapun, kumpulan dos rendah tidak menunjukkan perbezaan bererti dengan kumpulan kawalan, maka pengambilan herba kacangma adalah dianggap selamat. Kajian ini mencadangkan tiada kesan ketoksikan sekiranya herba ini diambil pada dos 0.5 g/kg berat badan seperti yang diperhatikan dalam kajian ketosikan sub-kronik 90 hari.

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