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Toxicological evaluation of dried kacangma *(Leonurus sibiricus)* in rats: I. Blood chemistry, body and organ weight changes

[Kajian ketoksikan kacangma kering *(Leonurus sibiricus)* pada tikus: I. Kimia darah, perubahan berat badan dan organ]

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Key words: *Leonurus sibiricus*, toxicity evaluation, blood chemistry, body and organ weight changes, *Sprague Dawley* rats

Abstract

Kacangma *(Leonurus sibiricus* L.*)* is a popular traditional herb that has been consumed for decades by the people of Sarawak as a herbal medicine or culinary ingredient. The toxicity of dried kacangma herb on *Sprague Dawley* male and female rats was evaluated through acute and sub-chronic studies. In the acute toxicity study (for 14 days), rats were given two dosages of kacangma i.e. 2.0 and 5.0 g/kg body weight. They were observed for any toxic signs especially death for the first 24 hours and continued up to 14 days. During the 14 days, none of the animals died and no significant differences were observed in body weight gain, food and water consumption.

Subsequently the sub-chronic toxicity was studied for 90 days. The rats were fed kacangma at the rate of 0.5 (low dose), 5 (medium dose) and 25 (high dose) g/kg body weight. The control groups of rats received only the commercial rat pellet. Minor treatment-related effects were observed for body weights, organ weights and the lipid profile parameters and these did not appear to be of toxicological significance. In the sub-chronic toxicity studies, some indications of renal and liver toxicity were evident in the medium and high dose groups when plasma creatinine and liver enzymes were found to be higher when compared with the control and the low dose groups. In conclusion, if the herb kacangma is consumed at the rate of 0.5 g/kg body weight, there is a less likely chance of developing toxicity as observed throughout the period of sub-chronic study.

Introduction

Kacangma *(Leonurus sibiricus)*, herbaceous shrub from Lamiaceae mint family; is a popular medicinal herb of Sarawak *(Plate 1)*. It is traditionally consumed as a folk medicinal herb for post-natal care, specifically to reduce body pain, as an emmenagogue and to hasten the involution of uterus after delivery (Chai et al. 1989). Due to its unique herbal flavour and aroma,

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*Plate 1. Kacangma herb (**Leonurus sibiricus** L.)*

kacangma is also widely used as a culinary ingredient (Teo and Chua 2001).

The role of kacangma as an under utilised herb with potential economic value has been recognised (MOA 1995; Paulus and Lau 2004). Subsequently, efforts are made to reassess and reevaluate its values; as well as increase its utilisation by developing into various special herbal products with commercial values.

Although many herbs have been used over the centuries and are generally considered as safe, there have been a number of recorded cases of intoxication with certain herbal products. For instance, high administrative doses of common spice herbs such as onion and garlic were found to cause toxic effects on liver and lungs (Alnaqeeb et al. 1996; Thomson et al. 1998). Prolonged period of consumption of other common traditional herbal preparations such as ginkgo, St. John Wort, ginseng, echinaceae and ephedra was also associated with side effects such as kidney injuries, adverse events of cardiovascular and central nervous systems (Kadiri et al. 1999; Haller and Benowitz 2000; Ernst 2002).

Although kacangma has been consumed for decades, the safety assessment of this Sarawak's *Leonurus* species has not been substantiated with scientific and clinical studies. Hence, the objective of the present study is to evaluate the long-term toxicity effect of dried kacangma herb in *Sprague Dawley* rats.

Materials and methods

Preparation of dried kacangma powder

Dried kacangma leaves were obtained by drying the freshly harvested kacangma herb planted in MARDI Kuching Station. The aerial parts of 2-month-old kacangma herb consisting of leaves and young stems were harvested, cleaned, finely chopped and oven-dried at 45 °C in a force-air oven until final moisture content was below 6% (w/w). The dried herb was then ground into powder and stored in airtight containers (Chua and Aminah 2003).

Preparation of formulated kacangma pellet

Formulated kacangma pellet was prepared by mixing the dried kacangma powder with a commercial rat pellet (Gold Coin Feedmills (M) Sdn. Bhd.). Water was added to bind the mixture. The paste-like mixture was reformed into pellet 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 pellet was then stored at chilled temperature in airtight containers to prevent mould growth. This processing method is shown in *Figure 1*. The ratio of dried herb calculated for each group was 0.5 (low dose), 5 (medium dose) and 25 (high dose) g/kg of body weight.

Experimental animals

A total of 56 *Sprague Dawley* rats (8 males and 8 females for acute oral toxicity, 20 males and 20 females for sub-chronic toxicity) each weighing between 180–205 g and approximately 70 days old, were

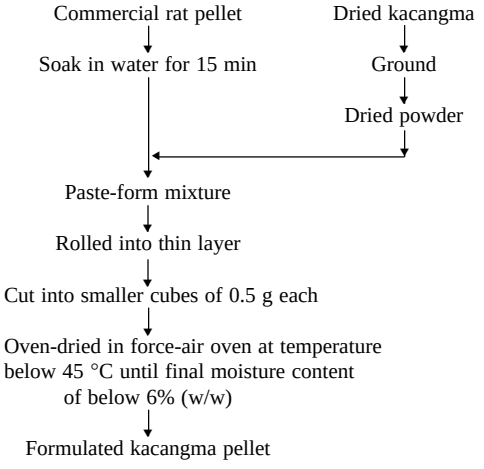

Figure 1. Preparation of formulated kacangma pellet

quarantined for about two weeks prior to initiation of the experiment. The rats were housed singly in elevated stainless steel cages and kept in a room, at temperature of 25–27 °C with a 12-h light/dark cycle. The rats were fed with formulated kacangma pellets and/or commercial rat feed and water *ad libitum.* Animal identification was via cages which were colour coded identification card indicating the animal and treatment groups. Litter paper placed beneath the cage was changed daily.

Animals were euthanasied with chloroform and the gross examination included the external surfaces, all orifices, all visceral organs, the cranial, thoracic, abdominal and pelvic cavities.

Acute oral toxicity

A single-dose acute oral toxicity (limit dose test) was conducted in rats to evaluate the potential toxicity of high exposure to kacangma herb. The rats were divided into two groups (8 rats per group, 4 males and 4 females). Each group was fed kacangma at the rate of 2 and 5 g/kg of body weight, respectively. Rats were then observed daily for mortality, signs of gross toxicity such as fur loss, frequent urination, diarrhoea and behavioural changes for the first 24 hours until the 14th day.

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. A total of 40 rats (20 males and 20 females) were equally distributed into four groups (10 rats per group, 5 males and 5 females). Group 1, 2 and 3 were fed 25 g formulated kacangma pellets each day with a dried kacangma content of 0.5 (low dose group), 5 (medium dose group) and 25 (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. Blood was collected in plain and EDTA coated vacuminated tubes from all rats at the end of the 90-day study prior to sacrifice. Rats were fasted over night prior to blood collection. Blood was evaluated for clinical biochemistry.

Body weight Body weights of all rats were recorded every 3 days during the experiment which included the initial and final stages of the experiment.

Organ relative weight After taking the blood samples for clinical pathology, the main organs viz. heart, kidneys, liver, lungs and pancreas were quickly excised of all excess tissues and fats and weighed immediately after rinsing in 0.9% cold saline to remove any blood. The organ relative weight (% body weight) was obtained by dividing the final weight of organ to final body weight.

Clinical biochemistry Approximately 2 ml of blood was collected from each rat via intracardiac puncture. The blood was transferred into plain and EDTA coated vacutainers. The serum fraction was analysed for total protein, albumin and enzyme activities: aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Lipid profile analysis included cholesterol, high-density

lipoprotein (HDL) and triglycerides levels; while urea and creatinine was evaluated for kidney function test. All analyses were performed using the AGII Chemical Analyser (Landmark Scientific Inc., North Carolina, USA).

Statistical analysis

Data were analysed using Analysis of Variance (ANOVA) at 5% level (p <0.05). Significance was determined using Duncan Multiple Range Test (DMRT) on all possible pairs of treatment means using the Statistical Analysis System (SAS). All values are expressed as group mean ± standard error of mean (SEM).

Results and discussion

Acute oral toxicity

Oral administration of dried kacangma herb did not induce any mortality even up to the highest dose, which was 5 g/kg body weight. All the treated rats also did not show any sign of gross toxicity, adverse pharmacological effects or abnormal behaviour throughout the observation period of 14 days. Under the conditions of this test, the acute oral toxicity dose for kacangma was greater than 5 g/kg.

Sub-chronic toxicity

The 90-day repeat dose oral study was conducted with daily administration of formulated kacangma pellet. Similar feed preparation method was used by Suhaila et al. (2001) in pegaga *(Centella asiatica)* efficacy test on *Sprague Dawley* rats. The dosage for the present study was formulated based on the normal human consumption of kacangma herb i.e. 0.5 g/kg body weight, as well as the contents of active constituents; alkaloid leonurine and stachydrine in *Leonurus* species (Yeung et al. 1977; Bradley 1992). Hence, dried kacangma at dose levels of 0.5, 5 and 25 g/kg body weight were selected for the study.

Body weight All the rats subjected to different kacangma supplementation showed an overall increase in body weights (male 275–295 g and female 210–225 g) as compared to the initial weights (male 190–205 g, female 180–190 g). These results were as expected because the rats were in the growing stage. Average overall body weights indicated that the treated rats regardless of the dose level were comparable to the controls *(Figure 2).* However, higher dose treated rats showed lower average body weight. This may be due to the presence of bitter substances in the kacangma pellet that could have limited the food intake by this group of rats. Increment in body weight is an important indication for health status of the experimental animals (Heywood 1983). Therefore, the result indicated a positive health status for both treated and control rats.

Organ relative weight Organ weight measurement is another important guide to assess general toxicity. Changes in organ weight are indicator of toxicity since organ weight will be affected by the suppression of body weight (Heywood 1983; Frank 1996). Although a statistically significant difference (p <0.05) was noted in final body weight for male and female rats, it was not significantly different (p >0.05) for organ relative weight between male and female rats as well as for treated and control groups *(Table 1).* This indicated organ relative weights were not affected by the administration of kacangma.

Clinical pathology There were no mortalities, signs of gross toxicity and behavioural changes in all the treated rats during the study. No significant gross abnormalities were also observed in the five main visceral organs.

Effects of kacangma on renal function Renal glomerular functions are assessable by measuring the plasma creatinine and urea concentrations (Moshi et al. 2001). Creatinine is a by-product from muscle as its concentration in blood will be affected by any changes in muscle mass.

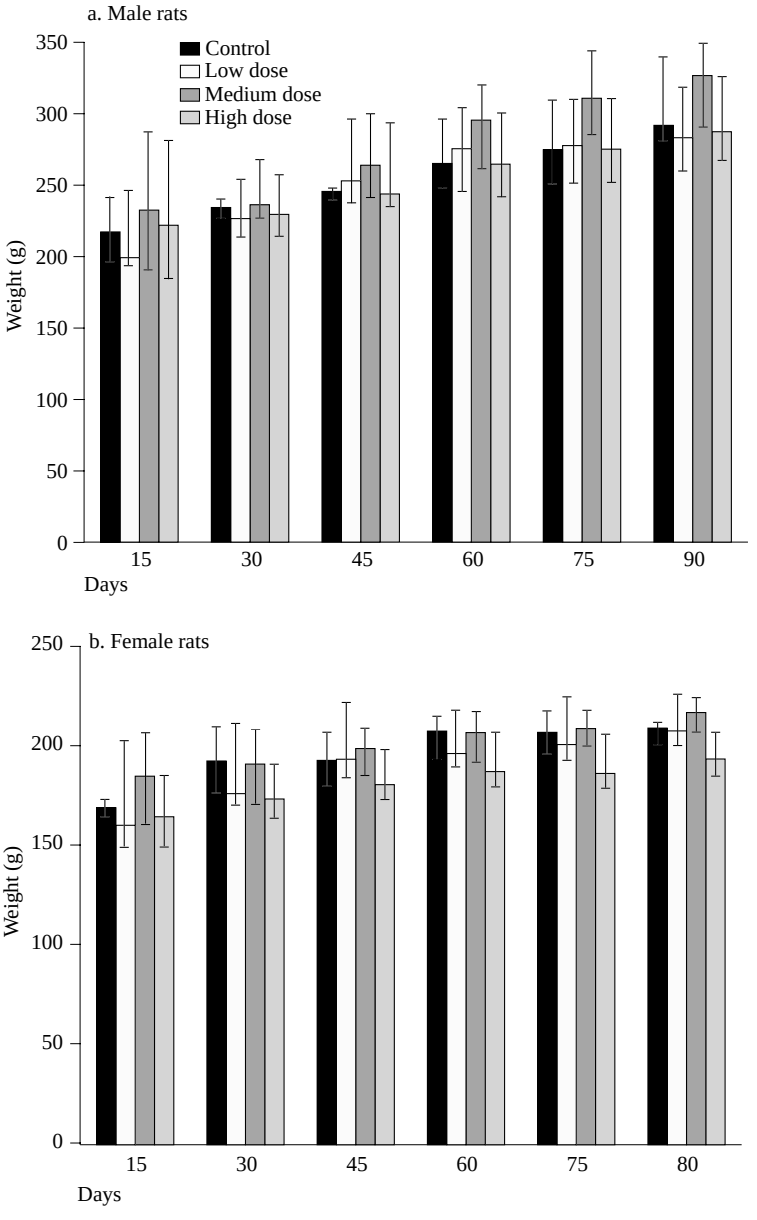

Figure 2. Effect of kacangma feed on body weights of (a) male rats and (b) female rats (value are mean ± SEM, n = 5)

Urea is synthesized in the liver as the primary by-product of deamination of amino acids (Vaughn 1999). Hence, increase in plasma creatinine or urea concentration would indicate renal damages.

There were no significant differences in urea concentrations between all groups of rats *(Table 2)*. However, the creatinine concentrations were significantly higher (p <0.05) in rats treated with medium and high doses of kacangma as compared to control and low dose treated rats. This indicates some injuries to the kidneys especially in the glomerular renal functions as a result of feeding with medium and high dose of kacangma to the rats.

Table 1. Effects of kacangma feed on body weight and organ relative weights in male and female rats (n = 5)

	Control		Low dose (0.5 g/kg)		Medium dose (5 g/kg)		High dose (25 g/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Body weight (g)								
Initial weight	200.00 ± 24.49a	190.00 ± 18.73a	190.00 ± 25.44a	188.00 ± 36.55a	205.00 ± 41.53a	180.00 ± 16.73a	198.00 ± 25.69a	185.00 ± 20.62a
Final weight	290.00 ± 37.42a	225.00 ± 27.24cd	295.00 ± 26.93a	210.00 ± 30.33d	275.00 ± 22.91abc	216.00 ± 14.97d	288.00 ± 33.11ab	220.00 ± 55.86d
Heart								
Weight (g)	0.86 ± 0.06	0.72 ± 0.01	0.95 ± 0.09	0.68 ± 0.09	0.83 ± 0.05	0.71 ± 0.05	0.86 ± 0.12	0.72 ± 0.04
% Body weight	0.30 ± 0.02a	0.32 ± 0.01a	0.32 ± 0.02a	0.33 ± 0.02a	0.30 ± 0.02a	0.33 ± 0.01a	0.30 ± 0.01a	0.33 ± 0.01a
Kidney								
Weight (g)	1.91 ± 0.40	1.40 ± 0.14	1.84 ± 0.22	1.19 ± 0.24	1.71 ± 0.17	1.41 ± 0.14	1.91 ± 0.15	1.52 ± 0.08
% Body weight	0.65 ± 0.06a	0.62 ± 0.06a	0.62 ± 0.05a	0.56 ± 0.05a	0.62 ± 0.06a	0.65 ± 0.06a	0.67 ± 0.08a	0.69 ± 0.07a
Liver								
Weight (g)	11.18 ± 1.56	7.54 ± 0.04	9.45 ± 2.40	6.62 ± 0.95	9.40 ± 0.46	7.20 ± 0.67	11.16 ± 0.69	8.51 ± 1.26
% Body weight	3.86 ± 0.26a	3.35 ± 0.02a	3.17 ± 0.57a	3.16 ± 0.11a	3.42 ± 0.08a	3.34 ± 0.28a	3.88 ± 0.36a	3.87 ± 0.44a
Lung								
Weight (g)	1.24 ± 0.05	1.23 ± 0.35	1.41 ± 0.28	1.16 ± 0.21	1.45 ± 0.28	1.22 ± 0.24	1.20 ± 0.19	1.21 ± 0.04
% Body weight	0.43 ± 0.05a	0.55 ± 0.14a	0.47 ± 0.08a	0.55 ± 0.10a	0.52 ± 0.08a	0.56 ± 0.09a	0.42 ± 0.03a	0.55 ± 0.04a
Pancreas								
Weight (g)	0.61 ± 0.02	0.42 ± 0.01	0.55 ± 0.07	0.48 ± 0.09	0.57 ± 0.05	0.48 ± 0.05	0.55 ± 0.07	0.48 ± 0.05
% Body weight	0.21 ± 0.02a	0.19 ± 0.01a	0.19 ± 0.03a	0.23 ± 0.02a	0.21 ± 0.01a	0.22 ± 0.01a	0.19 ± 0.02a	0.22 ± 0.02a

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

Table 2. Effects of kacangma feed on plasma creatinine and urea concentrations (renal function) in male and female rats (n = 5)

	Control		Low dose (0.5 g/kg)		Medium dose (5 g/kg)		High dose (25 g/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Creatinine (µmol/litre)	41.80 ± 2.13a	44.60 ± 2.54a	41.80 ± 3.33a	42.27 ± 1.73a	55.13 ± 5.25b	53.33 ± 4.55b	50.40 ± 4.40b	53.00 ± 6.36b
Urea (mmol/litre)	12.16 ± 0.21a	11.67 ± 0.42a	12.73 ± 0.77a	13.66 ± 2.16a	12.79 ± 0.71a	13.16 ± 1.40a	12.22 ± 0.62a	12.00 ± 0.82a

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

Effects of kacangma on plasma lipid profile There were also no significant differences ($p > 0.05$) seen in all the plasma lipid profiles in the treated groups as compared to the control. This indicates that the concentrations of plasma cholesterol, HDL and triglycerides were not affected by the consumption of kacangma in all rats *(Table 3).*

Effects of kacangma on liver function The serum chemistry and enzyme activities in kacangma fed rats are shown in *Table 4*. The ALT level was significantly higher in medium and high dose groups when compared to the control and the low dose group. ALT is an indicator of liver necrosis in small mammals (Cornelius 1989). On the other hand, there was a slight decrease in ALP levels in the medium and the high dose groups.

Conclusion

Kacangma showed no noticeable gross toxicity in acute oral test, indicating that even at high dose it did not cause death in the rats. In the sub-chronic toxicity study, minor treatment-related effects were observed for body weights, organ weights and the lipid profile parameters and these did not appear to be of toxicological significance. However, there were some indications of renal and liver toxicity evident in the medium (5 g/kg body weight) and high dose (25 g/kg body weight) groups when plasma creatinine and liver enzymes were found to be higher when compared with the normal dose. In conclusion, if the herb kacangma is consumed at the rate of 0.5 g/kg body weight, there is likely a less chance of developing toxicity as shown in the sub-chronic toxicity study.

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Table 3. Effects of kacangma feed on plasma cholesterol, HDL and triglycerides (lipid profile) in male and female rats (n = 5)

	Control		Low dose (0.5 g/kg)		Medium dose (5 g/kg)		High dose (25 g/kg)	
Male	Female	Male	Female	Male	Female	Male	Female	Male
Cholesterol (mmol/litre)	1.96 ± 0.16a	1.65 ± 0.12a	1.83 ± 0.03a	1.88 ± 0.08a	1.93 ± 0.09a	1.92 ± 0.06a	1.87 ± 0.13a	1.90 ± 0.11a
HDL (mmol/litre)	1.56 ± 0.13a	1.43 ± 0.09a	1.54 ± 0.21a	1.31 ± 0.12a	1.52 ± 0.07a	1.38 ± 0.07a	1.36 ± 0.06a	1.36 ± 0.09a
Triglycerides (mmol/litre)	1.19 ± 0.10a	1.07 ± 0.20a	0.93 ± 0.22a	1.33 ± 0.14a	1.03 ± 0.13a	1.22 ± 0.14a	1.04 ± 0.17a	0.93 ± 0.12a

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

Table 4. Effects of kacangma feed on serum chemistry and enzyme activities (liver function) in male and female rats (n = 5)

	Control		Low dose (0.5 g/kg)		Medium dose (5 g/kg)		High dose (25 g/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Total bilirubin (µmol/litre)	2.74 ± 0.68a	2.74 ± 0.42a	2.74 ± 0.42a	3.42 ± 0.54a	3.42 ± 0.54a	3.76 ± 0.34a	3.76 ± 0.64a	2.74 ± 0.42a
Protein (g/dl)	6.70 ± 0.19a	6.86 ± 0.10a	6.98 ± 0.27a	6.72 ± 0.20a	6.56 ± 0.22a	6.74 ± 0.29a	6.90 ± 0.27a	6.42 ± 0.27a
Albumin (g/dl)	3.86 ± 0.10a	3.76 ± 0.05a	3.92 ± 0.14a	3.96 ± 0.07a	3.84 ± 0.07a	3.88 ± 0.15a	3.90 ± 0.17a	4.04 ± 0.14a
Globulin (g/dl)	3.00 ± 0.13a	2.98 ± 0.20a	2.98 ± 0.18a	2.84 ± 0.10a	2.70 ± 0.21a	2.78 ± 0.16a	2.66 ± 0.18a	2.70 ± 0.17a
A/G Ratio	1.30 ± 0.08a	1.28 ± 0.12a	1.34 ± 0.08a	1.44 ± 0.06a	1.46 ± 0.14a	1.40 ± 0.07a	1.50 ± 0.16a	1.54 ± 0.12a
Aspartate transaminase AST (µ/litre)	384.80 ± 75.72a	342.40 ± 42.43a	369.00 ± 31.40a	366.80 ± 13.03a	432.80 ± 36.79a	434.00 ± 48.85a	432.60 ± 109.50a	487.20 ± 37.71a
Alanine transaminase ALT (µ/litre)	105.40 ± 13.40a	91.00 ± 10.75a	101.20 ± 2.48a	87.40 ± 5.57a	156.00 ± 27.98b	153.80 ± 48.79b	143.60 ± 26.78b	144.40 ± 12.45b
Alkaline phosphatase ALP (µ/litre)	126.00 ± 4.25a	124.60 ± 13.12a	125.20 ± 9.07a	115.40 ± 16.53ab	99.20 ± 2.58bc	94.60 ± 14.99bc	97.20 ± 6.66bc	87.20 ± 17.06c

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

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Abstrak

Kacangma *(Leonurus sibiricus* L.*)* ialah sejenis herba tradisional yang sudah sekian lama digunakan oleh penduduk Sarawak sebagai herba ubatan dan ramuan masakan. Ketoksikan kacangma kering terhadap tikus *Sprague Dawley* jantan dan betina telah dinilai melalui kajian akut dan sub-kronik. Dalam ujian akut (14 hari), tikus telah diberi makan dua dos kacangma iaitu 2 dan 5 g/kg berat badan. Kesemua tikus diperhatikan tanda-tanda ketoksikan terutamanya kematian dalam masa 24 jam sehingga 14 hari. Dalam tempoh 14 hari, tiada kematian dan tiada perbezaan bererti diperhatikan pada berat badan, pengambilan makanan dan air.

Berikutan ini, kajian sub-kronik selama 90 hari dijalankan. Tikus diberi makan kacangma pada kadar 0.5 (dos rendah), 5 (dos sederhana) dan 25 (dos tinggi) g/kg berat badan. Tikus kawalan hanya diberi pelet komersial. Kesan perlakuan yang minor telah didapati pada berat badan, berat organ dan parameter profil lipid dan kesemua ini tidak menunjukkan kesan yang bererti dari segi toksikologi. Dalam kajian sub-kronik, sedikit pertanda ketoksikan telah dikesan pada renal dan hati untuk kumpulan dos sederhana dan tinggi apabila aras kreatinin plasma dan enzim-enzim hati didapati lebih tinggi berbanding dengan kumpulan kawalan dan kumpulan dos rendah. Sebagai kesimpulan, sekiranya herba kacangma diambil pada aras 0.5 g/kg berat badan, maka tiada kesan ketoksikan ditemui seperti yang diperhatikan dalam kajian sub-kronik selama 90 hari.

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