

Haematological and histopathological evaluation of dried kacangma (*Leonurus sibiricus*) in New Zealand White rabbits

(Penilaian hematologi dan histopatologi kacangma kering (*Leonurus sibiricus*) pada arnab *New Zealand White*)

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Abstract

Kacangma (*Leonurus sibiricus* L.) is a popular traditional herb that has been consumed for decades by the people of Sarawak as herbal medicine and culinary ingredient. This study was conducted to evaluate the toxicity of kacangma herb on *New Zealand White* male and female rabbits through subchronic studies. Effects of kacangma herb intake at the level of 0.5, 5.0 and 25.0 g/kg body weight was evaluated for 90 days with focus on hematological and histopathological studies. The haematological study revealed no significant changes in all parameters studied i.e. haemoglobin, red blood cell value, white blood cell value, packed cell volume, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration. Vice versa, in the histopathological study, administration of dried kacangma herb at medium and high dose was found to cause adverse effects on histopathological structure in liver and kidney of both male and female rabbits. However, since low dose group showed no significant differences to the control group, therefore it is considered safe and less chance of developing toxicity if the herb is consumed at the low dose of 0.5 g/kg body weight as observed throughout the 90 days period of subchronic study.

Keywords: *Leonurus sibiricus*, toxicity evaluation, haematology, histopathology, *New Zealand White* rabbits

Introduction

Kacangma (*Leonurus sibiricus*), a herbaceous shrub from Lamiaceae mint family; is a popular traditional plant which has been consumed for decades by the people of Sarawak as 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 recognised (MOA 1995; Paulus and Lau 2004). Efforts are made to reassess and reevaluate its values; as well as increase its

utilisation by developing into various specialty herbal products with commercial significance (Chua et al. 2014).

Safety is regarded as one of the three important aspects in herbal products besides quality and efficacy. 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. Several reports indicated that prolonged period of traditional herb consumption such as ginkgo, St. John Wort, ginseng, echinaceae and ephedra were associated with toxicity on kidney, liver, lung, cardiovascular and central nervous

system (Alnaqeeb et al. 1996; Thomson et al. 1998; Kadiri et al. 1999; Haller and Benowitz 2000; Ernst 2002). Consequently, it is very important to understand the potential side effect of traditional herbs besides their health benefits.

Dried kacangma herb has been consumed for many generations and is generally considered as safe and harmless mainly due to its natural origin without extensive substantiate by sufficient scientific and clinical studies (Chua and Aminah 2013). Previous 90-day subchronic toxicity study of dried kacangma has been carried out on blood chemistry, body and organ weight changes in *New Zealand White* rabbits (Chua et al. 2007). Therefore, this study was conducted as continuation to further evaluate the safety of dried kacangma herb with focus on haematology and histopathology.

Materials and methods

Preparation of dried kacangma powder

Dried kacangma leaves were obtained by drying the freshly harvested kacangma herb planted in MARDI Station Kuching. 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 pellet (Gold Coin Feedmills (M) Sdn. Bhd.). This processing method is shown in *Figure 1*. 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.

The pellet was formulated into three groups based on the ratio of dried kacangma herb ie. low dose, medium dose and high dose. 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 group was formulated based on the normal dose use of kacangma herb in human consumption i.e. 0.5 g/kg of body weight, as well as the homeopaths' recommended level of active alkaloid constituents in most *Leonurus* species (Yeung et al. 1977; Bradley 1992). The medium dose and high dose were respectively calculated as 10 and 50 times of low dose (WHO 1993).

Since the weight of rabbits are much lower as compared to human, the dose level of kacangma has been calculated based on the ratio average body weight of human vs. rabbits ie. 0.5 (low dose), 5.0 (medium dose) and 25.0 (high dose) g/kg of body weight.

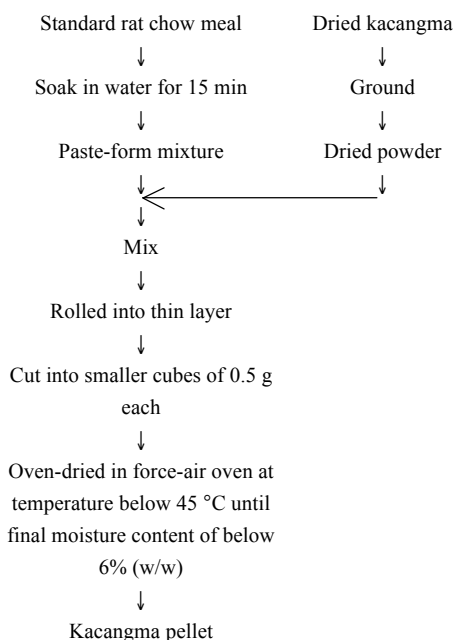


Figure 1. Processing of kacangma pellet

Experimental animals

A total of 32 *New Zealand White* rabbits (16 males and 16 females) each weighing average 750 g were acclimatised for 2 weeks before starting the study. According to the guidelines for toxicity investigation of herbal products using rabbits (non-rodents) as experimental animals, at least three animals per group per sex should be used for long-term (subchronic and chronic) toxicity test to be statistically validated (WHO 1993). The rabbits were housed individually in elevated stainless steel cages and kept at temperature of 25 °C with a 12-hour light/dark cycle. The rabbits were provided with formulated kacangma pellets and water *ad libitum* (free access for 24 hours).

A 90-day repeat dose oral study was conducted to evaluate the potential toxicity of kacangma herb. The rabbits were equally distributed into four groups (8 rabbits per group, 4 males and 4 females). Group 1, 2 and 3 were fed formulated kacangma pellets

each day with a dried kacangma content of 0.5 (low dose), 5.0 (medium dose) and 25.0 (high dose) g/kg of body weight, respectively for 90 days. Group 4 served as a control and was fed only commercial rat pellet. Animal identification was via cages; which were colour-coded identification card indicating the animal and treatment groups. At the end of the experiment, all the rabbits were fasted overnight for at least 15 hours and euthanased with chloroform.

Haematology studies

After 90 days of feedings, all rabbits 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 (Abbot Diagnostic Division, USA).

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

Histopathological examination

The histopathological examination was conducted according to method described by Luna (1986) and Dioka et al. (2002). After taking the blood samples for haematology studies, all rabbits were killed by cervical dislocation under chloroform anaesthesia and necropsied. The five main organs included kidney, liver, lung, heart and pancreas were quickly excised and weighed immediately 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 listed above were fixed in 10% neutral buffered formalin for analysis. Histopathological examination was carried out at Histopathology Laboratory, Faculty of Veterinary, UPM.

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 \pm standard error of mean (SEM).

Results and discussion

Haematology

The effect of kacanggma feed on haematology values in male and female rabbits are shown in *Table 1*. Haematology study has been recognised as an easy and readily screen for haematotoxicity (Goldstein 1988). 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 effects cause by the administration of drugs or herbal preparations (Halimah 2001).

Red blood cell volue (RBC) is studied for disorders related to abnormalities in the number of circulating cells ie. anaemia (severe reduction) or polycythemia (severe increase), whereas white blood cell value (WBC) is studied for disorders either leucopenia (severe reduction) or leukocytosis (severe increase) (Fishbach 1980). In this study, the results showed that there was no significant difference in the RBC and WBC values in the treated rabbits as compared to the control group. There were also no signs of anemia, polycythemia, leucopenia as well as leukocytosis occurred in all treated and control animals.

The measurement of haemoglobin (HGB) and packed cell volume (PCV) were also important in toxicology study since both parameters are related to the concentration of RBC. The HGB 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 HGB normally occurred in anaemia, hemorrhage or severe bleeding cases. Meanwhile, PCV or so-called haematocrit value is a measure in percentage of the relative volume of plasma, the total red blood cell mass and the concentration of red blood cells. A decrease in PCV will indicate anemia or leukemia (Vaughn 1999). Result indicated that there were no significant changes in both HGB and PCV values in all treated and control rabbits.

Mean corpuscular volume (MCV) indicates average red blood cell size, while mean corpuscular haemoglobin (MCH) refers to haemoglobin amount per red blood cell. Mean corpuscular haemoglobin concentration (MCHC) which is calculated by dividing HGB by PCV, refers to haemoglobin concentration per red blood cell, or haemoglobin amount relative to the size of the cell. In this study, no significant differences were observed in MCV, MCH and MCHC in all groups of rabbits.

Table 1. Effects of kacangma feed on hematological values in male and female rabbits (n = 4)

	Control		Low dose (0.5 g/kg)		Medium dose (5.0 g/kg)		High dose (25 g/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Haemoglobin concentration (HGB) (g/dl)	13.55 ± 0.15a	13.50 ± 0.20a	13.78 ± 1.31a	13.47 ± 1.16a	12.50 ± 0.51a	14.23 ± 0.45a	13.73 ± 1.21a	13.60 ± 1.19a
White blood cell (WBC) (mm ³)	7800.00 ± 800.00a	7900.00 ± 700.00a	7325.00 ± 1972.10a	7100.00 ± 1920.07a	7066.67 ± 917.73a	8000.00 ± 1344.12a	7825.00 ± 2606.88a	6566.67 ± 679.87a
Red blood cell (RBC) (10 ¹² /l)	5.35 ± 0.75a	5.80 ± 0.10a	6.23 ± 0.17a	6.23 ± 0.17a	5.33 ± 0.29a	5.83 ± 0.21a	5.88 ± 0.71a	5.80 ± 0.70a
Packed cell volume (PCV) (%)	36.50 ± 0.50a	36.75 ± 0.50a	39.00 ± 1.15a	39.33 ± 0.94a	34.33 ± 2.36a	40.67 ± 3.86a	37.75 ± 2.36a	37.67 ± 2.36a
Mean corpuscular Volume (MCV) (fl)	66.25 ± 9.54a	63.50 ± 0.50a	63.00 ± 3.16a	63.67 ± 2.87a	69.00 ± 8.52a	63.67 ± 0.47a	62.75 ± 2.06a	63.33 ± 1.70a
Mean corpuscular haemoglobin (MCH) (pg)	24.75 ± 3.59a	23.50 ± 0.50a	21.75 ± 2.50a	21.67 ± 2.49a	25.33 ± 3.30a	23.33 ± 0.47a	23.75 ± 0.50a	24.00 ± 0.25a
Mean corpuscular haemoglobin concentration (MCHC) (%)	35.00 ± 3.00a	36.50 ± 0.50a	35.33 ± 2.08a	35.00 ± 2.00a	35.67 ± 1.89a	36.67 ± 0.94a	37.75 ± 0.50a	37.67 ± 0.47a

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

Histopathology

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

However, 90-day intake of kacangma herb at medium and high dose was found to cause a degree of histopathological changes randomly in liver of both male and female rabbits. On the other hand, no abnormalities of liver were observed in control and low dose groups. Therefore, it can be concluded that administration of kacangma herb was found to have a tendency to affect the liver at medium and high dose levels.

Changes of histopathological structure in liver of rabbits at medium and high dose groups included billiary hyperplasia and degenerative of hepatocyte cells (*Plate 1*). 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 are involved in the intake, biotransformation and elimination of these potentially toxic compounds (Guillouzo 1998).

The histopathological study also revealed that all rabbits in medium and high dose group of kacangma intake showed renal nephrosis. According to Moshi et al. (2001), kidney is the second organ most frequently affected by toxic compound. This indicates some injuries to the kidneys especially in the glomerular renal functions as a result of kacangma intakes at medium and high doses.

No abnormalities of kidney were observed in control and low dose groups. Hence, it is considered less likely chance of developing toxicity of kidney if kacangma is consumed at the low dose of 0.5 g/kg body weight as observed throughout this subchronic study.

Conclusion

The haematological study of dried kacangma intake in *New Zealand White* rabbits revealed no significant changes in all parameters studied. However, administration of kacangma at medium and high dose was found to cause a degree of histopathologic changes in liver and kidney of both male and female rabbits. Since low dose group (0.5 g/kg body weight) showed no significant differences as compared to the control group, hence kacangma were considered safe as observed throughout the 90 days period of subchronic study.

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Table 2. Histopathological examination of five main organs in male and female rabbits (n = 4)

Dose		Liver	Kidney	Lung	Heart	Pancrease
Control	Male	All showed NA	All showed NA	All showed NA	All showed NA	All showed NA
	Female	All showed NA	All showed NA	All showed NA	All showed NA	All showed NA
Low dose (0.5 g/kg)	Male	All showed NA	All showed NA	All showed NA	All showed NA	All showed NA
	Female	All showed NA	All showed NA	All showed NA	All showed NA	All showed NA
Medium dose (5.0 g/kg)	Male	<ul style="list-style-type: none"> • 3 showed NA • 1 showed mild degeneration biliary hyperplasia and megalocytosis lymphocytic infiltration, with a lot of kupfer cells in the sinusoid of the liver 	All showed renal nephrosis	All showed NA	All showed NA	All showed NA
	Female	<ul style="list-style-type: none"> • 3 showed NA • 1 showed mild degeneration biliary hyperplasia and megalocytosis lymphocytic infiltration, with a lot of kupfer cells in the sinusoid of the liver 	All showed renal nephrosis	All showed NA	All showed NA	All showed NA
High dose (25.0 g/kg)	Male	<ul style="list-style-type: none"> • 1 showed NA • 3 showed severe degeneration biliary hyperplasia with bridging and degenerative of hepatocyte cells 	All showed renal nephrosis	All showed NA	All showed NA	All showed NA
	Female	<ul style="list-style-type: none"> • 2 showed NA • 2 showed severe degeneration biliary hyperplasia with bridging and degenerative of hepatocyte cells 	All showed renal nephrosis	All showed NA	All showed NA	All showed NA

NA = No abnormalities in histopathological structure

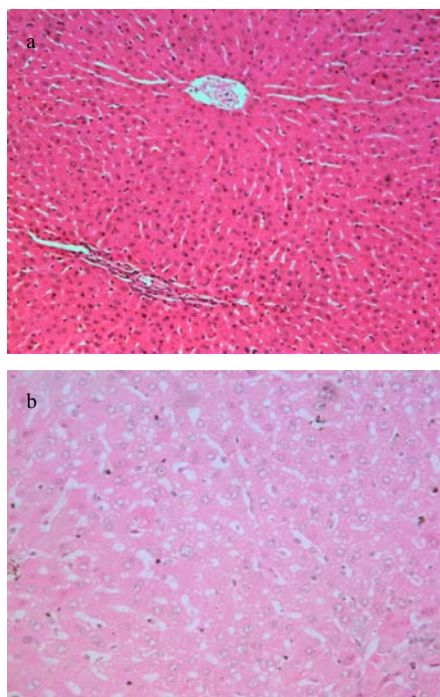


Plate 1. Representative photographs (40x) of liver sections from the control and high dose groups stained with hematoxylin-eosin.
 (a) Normal liver histopathological structure.
 (b) A medium-dose treated group showing liver with mild degeneration biliary hyperplasia.

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Abstrak

Kacangma (*Leonurus sibiricus* L.) adalah sejenis herba tradisional yang telah lama digunakan oleh penduduk Sarawak sebagai herba ubatan dan ramuan dalam masakan. Kajian ini telah dilakukan untuk menilai ketoksikan kacangma kering terhadap arnab jantan dan betina *New Zealand White* melalui kajian ketoksikan subkronik. Kesan pengambilan kacangma kering pada aras 0.5, 5.0 dan 25.0 g/kg berat badan terhadap arnab ditentukan selama 90 hari dengan tumpuan pada kajian hematologi dan histopatologi. Kajian hematologi menunjukkan tiada perubahan bererti pada kesemua parameter yang dikaji iaitu nilai hemoglobin, sel darah merah, sel darah putih, isi padu pek sel, isipadu sel min, hemoglobin sel min dan pekatan hemoglobin sel min. Dalam kajian histopatologi pula, pengambilan herba kacangma pada dos sederhana dan tinggi didapati menyebabkan perubahan bererti pada struktur histopatologi hati dan ginjal tikus jantan dan betina. Walau bagaimanapun, oleh kerana kumpulan dos rendah tidak menunjukkan perbezaan bererti dengan kumpulan kawalan, maka pengambilan herba kacangma adalah dianggap selamat dan tidak menyebabkan ketoksikan sekiranya herba ini diambil pada dos 0.5 g/kg berat badan sebagaimana yang diperhatikan dalam kajian ketoksikan subkronik 90 hari.