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ACUTE TOXICITY TEST OF THE COMBINED EXTRACT OF GAMBIR AND SECANG ON RATS

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ABSTRACT

The combined extract of g*a*mbir (*Uncaria gambir* (Hunter) Roxb.) and secang (*Caesalpinia sappan* L.) (formula) had been proven to demonstrate lowering uric acid activity in vivo. This study was aimed to evaluate acute toxicity of this combined extract on rats. The test was carried out based on The Guideline issued by The Indonesian National Agency of Drugs and Foods Controls (Badan POM). For this purpose, 30 females and 30 males *Sprague dawley* strain rats were divided into 6 groups, each group consisted of 5 pairs of sex, namely 5 treatment groups and normal group. Samples suspended in water solution 0.5% CMC were gavaged once per orally (p.o.) and then these animals were assessed for clinical signs, body weight and number of mortality for 14-day period. The LD50 (lethal dose-50) value was determined using a linier equation of Microsoft Excel program. The results showed that the formula had LD50 value more than 15 mg/kg body weight (b.w.) without some toxic symptoms and the body weight changes of each group were not altered significantly. It may be concluded that formula was in the practically non-toxic category without any serious toxic symptoms.

KEYWORDS: Acute toxicity test, Gambir (*Uncaria gambir* (Hunter) Roxb.), Secang (*Caesalpinia sappan* L.), Rat, LD50 value.

INTRODUCTION

Hiperuresemia is one of metabolic disorders characterized by high blood-uric acid level. The prevalence of hiperuresemia tends to rise year by year in line with the increase of economic status.[1] Hiperuresemia can lead to arthritis gout in addition to other secondary diseases.

Purin substances are metabolized by xanthin oxidase (XO) enzyme and produce uric acid and other free radical compound such as superoxide anion and H2O2, and then the radicals can destroy membrane lipid cell cause damage and cell death.[2] Therefore, controlling the XO activity is important not only to prevent the hyperuricemia but also to anticipate the emergence of other secondary illnesses.

Previous studies demonstrated that natural products contained high flavonoids had antioxidant effects and exhibited the uric acid-lowering activity as well.[3,4] Gambir or *Uncaria gambier* belongs to Rubiaceae family is an Indonesian native plant[5] withflavonoid content of it water extract is around 40-80% including (+) catechin.[6] Antioxidant activity of Gambir had previously been studied in vitro and in vivo.[7,8,9]

Secang known as *Caesalpinia sappan* L. (*Sappan wood*) which belongs to Leguminose family is one of the medicinal plant that have been utilized for diseases treatment of diseases such as antidiabetic, antiinflamation and industrial purposes as dye material.[10] Both extract and chemical compound of sappan had been reported showing antioxidant and antihyperuricemia effects. Previous studies stated that XO inhibitory activity was contributed by chemical compound such as neosappanone A, a dimer methanodibenzoxocinone, of which the activity is dose dependent and competitively inhibited with IC50 value of 29.7 uM and Ki 16.3 uM.[11] Some compounds isolated from the MeOH extract proved inhibiting xanthine oxidase, namely, neoprotosappanin, protosappanin, protosappanin E-2, sappanchalcone.[12] Methanolic extract of secang inhibited XO with IC50 value less than 20 ppm.[13] These compounds as stated above demonstrated antioxidant property with mechanism such as scavenging free radicals, interupting the oxidative chain and preventing radical formation.[12]

The proportional extract combination of gambir and secang had been reported to inhibit XO activity in vitro and in vivo.[14] Natural product that could inhibit XO activity and antioxidant has a strategic value in the development of a new drug to treat hyperuricemia. Beside the efficacy evidence, the scientifically safety evaluation for a new drug or a new combined extract is needed. Thus, this study aimed to evaluate the oral acute toxicity of the combined

extract to obtained information related the LD_{50} value, clinical symptoms and body weight change.

MATERIALS AND METHOD

Materials

Fresh gambir (leaves and twigs) were collected from Limapuluh Kota – West Sumatera Province on February 2015. Sappan wood was from the personal garden in Purwakarta-West Java on March 2015. Both plants were identified in the Research Center for Biology Indonesian, Institute of Science (LIPI) Bogor-West Java, before being processed.

Animals used were white *Sprague Dawley* strain rat female and male, 6-8 week old, 100-150 g bw for male and 100-125 g bw for female, that were obtained from Badan POM. Five rats of the same sex were housed in one polycarbonate cage with free access to commercial pellet food and tap water The room experiment was maintained with 12:12 h light/dark cycle, temperature at 22 ± 3^0C, and room humidity 30%–60%.

METHODS

Preparation of extracts

Gambir extract was prepared with maceration method. Dry powder gambir leaves was macerated using ethanol as eluent at room temperature for 12 hours with agitation. The collected filtrate was then separately evaporated under vacuum at 45^0C until semisolid mass obtained. Secang extract was produced by the similar procedures mentioned above. Formula was a homogeny proportional combination of both extracts.

Acute toxicity study

This study was conducted based on The Guindeline of Indonesia National Agency of Drug and Food Control.[15] The animal protocol had been reviewed and approved by Health Research Ethic Commiittee of Health Department Medicine University Indonesia Cipto Mangunkusumo Hospital, Indonesia (notification number 712/UN2.F1/ETIK/2015).

After acclimatization, 30 rats of each sex were divided into 6 groups randomly, namely, five treatment groups, DOSE-1, DOSE-2, DOSE-3, DOSE-4 and DOSE-5 receiving formula at dose of 0.938; 1.875; 3.750; 7.500; 15.000 g/kg bw, respectively, and one normal group treated with carrier in the same manner. Formula was suspended in 0.5% CMC solution with volume of treatment 1 mL/100 g bw. Before treatment, animals were fasting 14-18 hours. All

doses were gavaged only once except the highest dose of which was given twice within three hours p.o. using syringe with blunt needle. General clinical signs of toxicity of each animal (physical symptom of central nerve system, autonomy nerve system and digestive system) were observed once per two hours for 6 hours after administration of the tested sample and daily thereafter for 14 days. Animals were free acceesed to food three hours after treatment. Body weight was recorded before sample administration (0) and again on day 3, 7, 10 and 14. The number of mortalities was monitored daily for the experimental period.

Data Analysis

The LD50 value was determined at the end of study using *Microsoft Excel* program. Evaluation of tested compound toxicity is based on Hodge and Sterner scale (Tabel 1).

Table 1: Hodge and Sterner toxicity scale.[15]

No	Term	LD_{50} (single dose to rat, oral) (mg/kg BB)
1	Extremely toxic	< 1
2	Highly toxic	1-50
3	Moderately toxic	50-500
4	Slighly toxic	500 – 5.000
5	Pratically non toxic	1.000-15.000
6	Relatively harmless	>15.000

RESULT AND DISCUSSION

Acute oral toxicity is toxicity tests to evaluate immediate toxic effects after administration of tested sample in large quantity with a single dose orally. Acute toxicity studies are commonly used to determine LD50 of drug or chemicals and natural products. LD50 is a dose that caused the mortality of 50% animal under determined condition.[16]

The observations of clinical signs were presented in Table 2. The reults showed that the administration of sample at all doses did not demonstrate any clinical of toxicology sigificantly as compared to those of normal group. In the first hours after the sample treatment, there were signs of the weakned motoric activity. But these conditions were recovered themselves after animals were maintained in the observation period. There were no treatment-related changes in the clinical signs of toxicity.

Table 2: Clinical observation of toxicity of each group.

Observation	Groups					
	Normal	Dose1	Dose2	Dose3	Dose4	Dose5
Central nerve system						
1. Sedation	ns	ns	ns	ns	ns	ns
2. Motoric activity	+/-	+/-	+/-	+/-	+/-	+/-
3. Convulsion	ns	ns	ns	ns	ns	ns
4. Tremor	ns	ns	ns	ns	ns	ns
Autonomic Nerve System						
1. Open eye	+/-	+/-	+/-	+/-	+/-	+/-
2. Salivation	ns	ns	ns	ns	ns	ns
3. Urination	ns	ns	ns	ns	ns	ns
Breath rate	+/-	+/-	+/-	+/-	+/-	+/-
Heart rate	+/-	+/-	+/-	+/-	+/-	+/-
Digestive system						
1. Diarrhea	ns	ns	ns	ns	ns	ns
2. Constipation	ns	ns	ns	ns	ns	ns
3. Bloody fesses	ns	ns	ns	ns	ns	ns
Stress hair	ns	ns	ns	ns	ns	ns

ns: No Symptom. +/-: Normal

Body weight of each group was recorded before treatment (Day 0) and on Day 3, 7, 10 and 14 after treatment as stated in Fig. 1. It was showed that average body weights of each group of sexes tended increase after oral treatment of tested sample, although there was a fluctuating pattern.

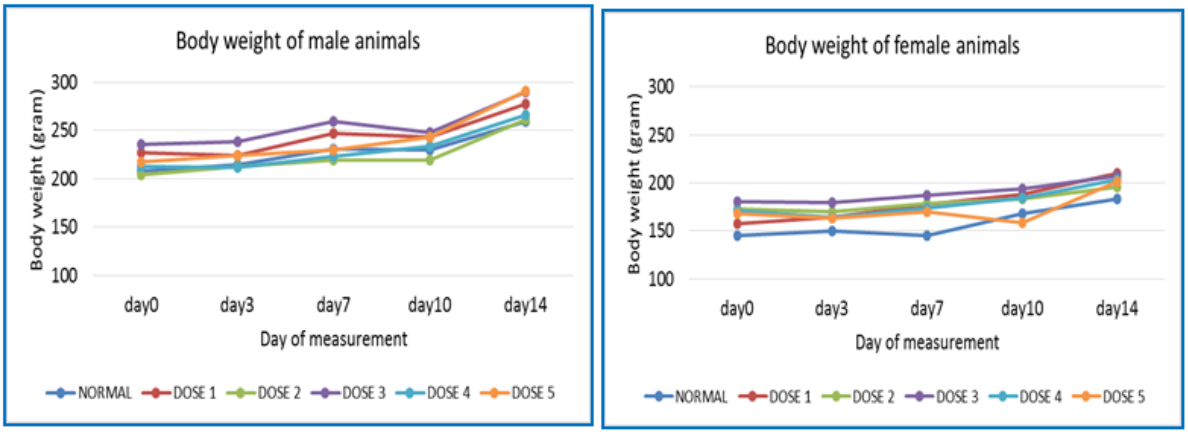

Figure 1: Average animal body weight of (a) male and (b) female rats during experimental period. N = 5 rats

The analysis toward the changes of body weight from day 0 against to day14 (Table 3) demonstrated that there were the different effects of orally administration tested sample on

both sexes. In male group, there was no decline percent body weight change even in the highest dose (Dose5 group) compared to normal group. Meanwhile, percent body weight on day14 in female groups exhibited decrease compared to normal group, especially animal administrated higher dose of tested sample. Female sex was more sensitive toward the tested sample than male one. However, statistically the percent changes of the body weights were found insignificant compared to normal group in both sexes ($p>0.05$). It meant that insignificant decrease in body weight of animal in each sex indicated that the administration of the combined extracts did not affect the growth of the animals.

Generally, body weight change is used as early indicator in the toxicity studies. Body weight loss of at least 10% indicates a toxic effect in which the tested material decrease animal's appetite The sign of body weight decrease which is an indication of elevating toxicity of extract/compound could be observed easily.[17]

Actually, medicinal plants exhibit natural properties. Body will tolerate these drugs as long as they are consumed appropriately and knowledgeably. The side effects will not show up significantly, as with synthetic drugs. Some various compounds sinergically worked each other with agonist mechanism.[18]

Table 3: Body weight change.

	Day0 (gram±sd)	Day14 (gram±sd)	Body weight change (gram)	% Weight change on day 14
Male				
Normal	208±17	259±7	51	24%
Dose 1	227±35	277±27	50	22%
Dose 2	204±27	261±23	57	28%
Dose 3	235±33	290±35	55	23%
Dose 4	213±6	266±17	53	25%
Dose 5	218±36	291±17	73	34%
Female				
Normal	146±15	184±15	38	26%
Dose 1	157±13	210±15	53	34%
Dose 2	173±12	196±12	24	14%
Dose 3	181±17	207±16	26	15%
Dose 4	171±9	203±11	32	19%
Dose 5	168±15	202±10	34	20%

Mean was average of 5 animals each group

The number of mortality of each treated and normal groups was depicted in Table 4.

Tabel 4: Number of animal mortality of each group.

Groups (g/kg bw)	Female	Male	% Mortality
Normal	0%	0%	0%
Dose1 (0.938)	0%	0%	0%
Dose2 (0.875)	0%	0%	0%
Dose3 (3.750)	0%	0%	0%
Dose4 (7.500)	10%*	0%	10%
Dose5 (15.000)	0%	10%**	10%

*on day 1. **on day 2.

Based on data on Table 4, administration of the tested sample did not cause the mortality of the experimental animals especially in three lower doses. In the two highest of treatment groups, namely Dose 4 dan Dose 5, occured animal mortalities by 10% in female (on day 1 after treatment) and male (on day 3 after treatment) group, respectively. After data analysis using linier equation of *Microsoft Excel* program, the LD_{50} value of tested sampel was more than 15 g/kg bw in which based on Hodge and Sterner Scale this compound was included to be relatively harmless category.[15] The LD_{50} value, defined as the statistically derived dose that when administered in an acute toxicity test expected to cause death in 50% of the treated animals in a given period, is currently the simple preliminary toxicity test and basis for toxicologic classification of chemicals.[19]

Acute oral toxicity test of both gambir and secang extracts separetely have been conducted previously and their results are in accordance to this study. It is reported that the acute toxicity test of the ethanolic secang extract on male and female rats that was administrated until the dose of 5000 mg/kg showed no toxicity at the some tested parameters such as general behavior change, mortality, or change in gross appearance of internal organs. In vitro study on fibroblast cell showed that both brazilin, the major compound of sappan, and brazilin rich extract were non-toxic at the concentration up to 500 ug/mL, respectively, during 24 hour of incubation. The viability of this cell was not altered by brazilin up to 100 uM during 24 hour incubation as well.[10]

Study acute oral toxicity of aqueous gambir extract had previously been done. Hasti S.[20] reported that the LC_{50} value of this aqueous extract was more than 15 g/kg bw so that based on Hodge and Sterner scale this extract was categorized as relatively harmless.[15] Cytotoxicity test on Vero normal cel line using 4,5-dimethylthiazol-2yl (MTT) method displayed that gambir leaves-derived ethanolic extract has the IC_{50} value of 700 ppm. It was

categorized as non-toxic extract when the IC_{50} value against to normal cell was more than 80 ppm.[21]

CONCLUSION

From the study, it could be concluded that the LD_{50} value of the proportionated combination of gambir and secang extract prepared in the study was more than 15 g/kg bw that was included to be relatively harmless category. Administration of the combine extract did not cause body weight change and did not demonstrate any clinical signs of toxicology significantly.

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