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Research Article

EFFECT OF THE KEPEL LEAVES EXTRACT (*STELECHOCARPUS BURAHOL* [BL.] HOOK. F. & TH.) ON SPRAGUE-DAWLEY RATS: AN ACUTE TOXICITY STUDY

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ABSTRACT

Objective: The aim of this study was to evaluate the acute effect of the Kepel leaves extract (*Stelechocarpus burahol* [Bl.] Hook. F. and Th.) after a single dose oral treatment on the body weight and the organ of Sprague-Dawley (SD) rats.

Methods: This experiment was conducted based on the OECD-guideline 401-1999. A single oral dose of the Kepel leaves ethanol extract (0, 200, 585, 1711, and 5000 mg/kg body weight) were given to male and female SD rats (each group, n=6). The control group was only given the vehicle (olive oil). The observation was done during 24 hrs and was continued until 14 days, because there was no death among the tested animals, included the recording of the death of animal test, body weight, toxic signs, duration, and reversibility of the toxic effect. A half of the rats in each group were sacrificed after 24 hrs observation, and remain of the animals were sacrificed after 14 days observation. The seven organs (kidney, lung, liver, spleen, intestine, stomach, and heart) were taken from each animal for macroscopic and microscopic (histopathology) examination.

Results: The results showed that the acute toxicity potency of the ethanol extract of Kepel leaves was practically non-toxic, and the pseudo-lethal dose of 50% (pseudo-LD_{50}) value was higher than 5000 mg/kg body weight.

Conclusion: Based on the macroscopic and microscopic observation, it could be concluded that the ethanol extract of Kepel leaves did not induce the toxic effect on the body weight and the organ of male and female SD rats.

Keywords: *Stelechocarpus burahol* (Bl.) Hook. F. and Th., Acute toxicity study, Body weight, Organ, Sprague-Dawley rat.

INTRODUCTION

Some studies about the potency of Kepel or *Stelechocarpus burahol* (Bl.) Hook. F. and Th. (*S. burahol*) as an anti-hyperuricemic agent have been reported. Susilowati [1] and Hening [2] evaluated the effect of the water extract of Kepel leaves in decreasing of plasma uric acid levels in rats and chicken, respectively. The anti-hyperuricemic activity of the soluble and not soluble fractions of petroleum eter on the chicken was reported by Sutomo [3]. Purwantiningsih [4] revealed that the ethanol and hexane leave extracts of Kepel leaves decreased the plasma uric acid levels on rats. The anti-hyperuricemic activity of the ethanol and hexane extracts was not significantly different as compared to the positive control of allopurinol. A study by Sunarni [5] has provided evidence that the isolated flavonoid from Kepel leaves has an antioxidant activity, and the xanthine oxidase inhibitors have been found in a wide variety of plants used in traditional herbal medicines for the treatment of gout and rheumatism [6,7].

Whereas Ariningsih [8] has performed an acute toxicity study of the ethanol extract of Kepel leaves on mice. The result showed that the ethanol extract was practically non-toxic, and the pseudo-lethal dose of 50% (pseudo-LD_{50}) was more than 5000 mg/kg body weight. Therefore, the Kepel leaves could be developed as phyto-pharmaceutical product for an anti-hyperuricemic agent. The Indonesian Government required a complete pre-clinical data for the registration of any phyto-pharmaceutical product intended for human use. One of the pre-clinical studies that required by the regulatory authority was the acute toxicity study. The preclinical data was required to ensure the safety, efficacy, and quality of the preparation.

Acute toxicity was described as the adverse effects of a substance that caused by either a single exposure [9] or multiple exposures in a short space of time (usually <24 hrs). The observation was done for 14 days after administration of the compound [10] involved the recording of toxic signs (e.g., behavior and body weight), duration, and reversibility of the toxic effect. An acute toxicity study for pharmaceutical development involved the administration of a single dose of the test substance and usually uses two different mammalian species, and two different routes. The routes of administration and species required vary according to the regulatory authority [11,12]. Different with the previous study which conduct acute toxicity tests using mice, the objective of this study was to evaluate the acute effect of the *S. burahol* leaves extract after a single dose oral treatment in different species, especially to determine the toxicity potency (LD_{50}) of the ethanol extract of *S. burahol* leaves and to evaluate the specific toxic effect on the body weight and organ of Sprague Dawley (SD) rats.

METHODS

Plant material

The *S. burahol* leaves were collected from Samigaluh area, Yogyakarta and authenticated in the Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Indonesia. The ethanol extract was made by maceration of dried leaves of Kepel with ethanol 96% for 24 hrs and re-macerate for 2 times. The mixture extract was concentrated by the evaporator, and then was extracted again by ethanol and was evaporated until was got the concentrated extract.

Animals

Male and female SD rats of age 2.5-3.5 months, weighing initially about 150-200 g, were kept in the animal house of Faculty of Pharmacy, Gadjah Mada University, Indonesia. This study was cleared by the Institutional Animal Ethical Committee. The animals were randomly divided into subgroups of five each and maintained under standard conditions. All animals were fed the standard rodent pellet (Comfeed, Indonesia) and water *ad libitum.*

Acute toxicity study

The acute toxicity study was performed based on the OECD-guideline 401-1999 (Organization for Economic Co-operation and Development).

The single oral doses of *S. burahol* leaves ethanol extract (0, 200, 585, 1711, and 5000 mg/kg body weight [b.w.]) were given to male and female SD rats (each group, n=10). The control group was only given the vehicle (olive oil). The observation was done during 24 hrs and was followed by 14 days observation because there was no death among the tested animals. A half of the rats in each group were sacrificed after 24 hrs observation, and remain of the animals were sacrificed after 14 days observation. The seven organs (kidney, lung, liver, spleen, intestine, stomach, and heart) were taken from each animal for macroscopic and microscopic (histopathology) examination. The rats were weighed every 3 days to determine average daily gain (ADG) (ADG = the body weight of rat at the end of day observation minus the initial body weight of rat and was divided by 14). The observation for 24 hrs and 14 days involved the recording of the death of animal test, toxic signs, duration, and reversibility of the toxic effect.

Data analysis

The number of animal death was recorded to determine the LD_{50}. The body weight data were presented as the mean ± standard error of the mean of the changes on the animal body weight and were evaluated by the Analysis of Variance and was followed by the Scheffe *post-hoc* test if there was a significant difference. The level of significance was set at $p<0.05$. The spectra of the specific toxic effects were evaluated based on the observation of toxic signs and the histopathology examination results.

RESULTS AND DISCUSSION

Acute toxicity is distinguished from chronic toxicity, which describes the adverse effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years). It is obviously unethical to test for acute (or chronic) toxicity in humans. However, some information can be gained from investigating accidental human exposures (e.g. factory accidents). Otherwise, most acute toxicity data comes from animal testing or, more recently, *in vitro* testing methods and inference from data on similar substances [9].

The acute effect of the *S. burahol* leaves ethanol extract has to be done as a consequence in the developing of the extract to be a phyto-pharmaceutical product. During the time of observation (24 hrs and 14 days), there was not found the animal death so that the potency of acute toxicity of the ethanol extract of *S. burahol* leaves in male and female rats could be classified as practically non-toxic and the pseudo-LD_{50} value was more than 5000 mg/kg b.w. [13,14]. Based on the physical observation, there was no change on the body weight of male and female rats as compared to the control group (Table 1) and there was not found the toxic-signs related with the central nervous system, respiratory, cardiovascular, genitourinary, digestion, skin and hair, eye, and mucosal membrane system. The statistical test results for the organ weight showed that the significant difference ($p<0.05$) was only found on the kidney weight of male rat group that was given the test preparation of 585 mg/kg b.w., and the stomach weight of female rat group after treated with the *S. burahol* leaves extract at doses of 1711 mg/kg b.w. as compared to control group, respectively (Tables 2 and 3). The macroscopic observation showed there was no change on the all of observed organs.

Table 1: Statistical analysis results of the ADG for the male and female rats during 24 hrs and were followed by 14 days observation

Group	ADG of the m ale rat groups (mean±SEM; n=5) g/day	ADG of the female rat groups (mean±SEM, n=5) g/day
I	−0.77±0.12	−0.36±0.33
II	−0.50±0.14	−0.05±0.22
III	−0.59±0.34	0.43±0.25
IV	−0.09±0.17	−0.33±0.08
V	−0.38±0.05	0.13±0.24

I: Control group; II-V: Treated groups at doses of 200, 585, 1711, and 5000 mg/kg body weight. p values of the ANOVA for male and female groups were more than 0.05. It means there was no significant difference of ADG among all groups, ADG: Average daily gain, SEM: Standard error of the mean, ANOVA: Analysis of variance

The microscopic examination was conducted on the histopathology preparations of seven important organs namely liver, kidney, spleen, lung, intestine, stomach, and heart. According to the histopathology, examination results could be evaluated that there were some changes in the 7 organs that were observed. They were congestion, inflammation, and hydropic degeneration in the liver, inflammation in the kidney, intestine, and stomach, while in the lung, spleen, and heart, there were no changes found.

The congestion was described as a condition that there was an excessive blood in the blood vessels in the certain areas and was also known as hyperemia. The tissues or hyperemia organ will be in the red or violet under visual observation, and the capillaries tissues will be fully filled by blood under microscopic observation. There were two mechanisms of the congestion, an increase of the amount of blood flowing into the area or a decrease of blood that flowed from the area. A congestion may occur in the inflammation areas [15]. The liver congestion was found in the male rat groups either during 24 hrs or 14 days observations. In the 24-hrs observation was found congestion in the control group (one case), in Group II (200 mg/kg b.w.) and IV (1711 mg/kg b.w.) were one case and two cases, respectively. While in the 14 days observation was found in the control group (4 cases) and Group II (one case). For female rat groups, congestion appeared in the 24 hrs observation in the groups were treated with 200 and 585 mg/kg b.w. of the extract with two and one cases, respectively. This was not found in all groups for 14 days observation. The congestion did not only occur in the organs of the treated animals but also appear in the control group, so it could be concluded that the congestion occurred was not caused by the test preparations.

The hydropic degeneration was found in the liver (Fig. 1). This was a cellular change that may be caused by metabolic disorders. The changes were reversible or irreversible depend on the cause of the injury [16]. Under microscopic examination, the cells undergoing hydropic degeneration showed a vacuole in the cytoplasm. The changes were caused by the accumulation of water in the cell. The injury in the cells

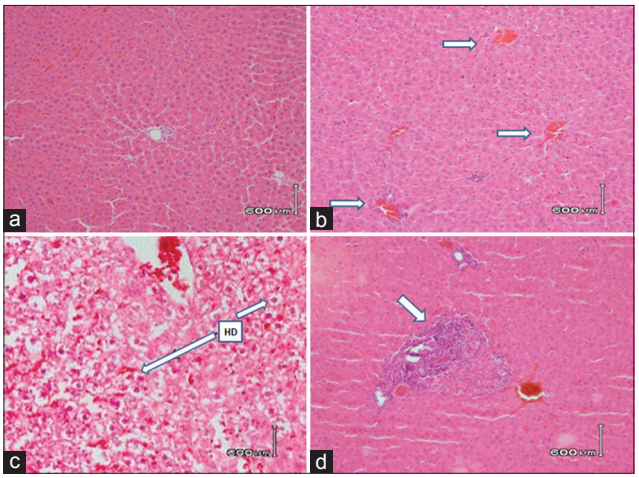

Fig. 1: Preview the liver histopathology in male rats after 24 hrs of the single oral dose treatment with the ethanol extract of Kepel leaves of 200-5000 mg/kg body weight. (a) Normal liver in control group, (b) liver with a congestion (was found in Group II), (c) liver with hydropic degeneration/HD (was found in Group IV), and (D) liver with lymphocytic infiltration in the inflammatory area (was found in Group V). Magnification of 10 × 40 and under hematoxylin eosin staining

Table 2: The organ weight of male rats groups after 14 days observation

Group	Liver	Stomach	Lung	Intestine	Spleen	Heart	Kidney
I	4.38±0.38	1.07±0.06	1.17±0.01	0.43±0.03	0.51±0.02	0.67±0.02	0.57±0.03
II	4.12±0.13	1.02±0.05	1.18±0.04	0.33±0.03	0.44±0.02	0.62±0.02	0.56±0.02
III	5.17±0.13	1.19±0.06	1.43±0.05	0.44±0.03	0.53±0.04	0.76±0.03	0.71±0.03*
IV	4.50±0.16	1.18±0.04	1.26±0.04	0.35±0.02	0.51±0.03	0.66±0.01	0.63±0.02
V	4.32±0.11	1.06±0.04	1.24±0.11	0.39±0.02	0.46±0.04	0.59±0.02	0.57±0.02

Data were presented as (mean±SEM) g, n=5; I: Control Group; II-V: Treated groups at doses of 200, 585, 1711, and 5000 mg/kg body weight. *p<0.05 significantly different as compared to the control group, SEM: Standard error of the mean

Table 3: The organ weight of female rats groups after 14 days observation

Group	Liver	Stomach	Lung	Intestine	Spleen	Heart	Kidney
I	4.19±0.17	1.12±0.04	1.34±0.08	0.38±0.02	0.43±0.02	0.53±0.01	0.45±0.03
II	4.09±0.08	1.29±0.06	1.15±0.04	0.35±0.02	0.41±0.03	0.53±0.02	0.43±0.01
III	4.08±0.09	1.21±0.04	1.06±0.05	0.38±0.02	0.47±0.04	0.54±0.01	0.41±0.01
IV	3.97±0.11	1.35±0.04*	1.09±0.05	0.37±0.05	0.44±0.03	0.53±0.01	0.44±0.01
V	4.30±0.31	1.23±0.05	1.16±0.08	0.45±0.02	0.41±0.05	0.60±0.02	0.43±0.02

Data were presented as (mean±SEM) g, n=5; I: Control Group; II-V: Treated groups at doses of 200, 585, 1711, and 5000 mg/kg body weight. *p<0.05 significantly different as compared to the control group, SEM: Standard error of the mean

resulted in the loss of volume settings and led to the morphological change that was called inflammation [15]. The hydropic degeneration was a common physiological mechanism due to the metabolic disruption that may be caused by a disease or lack of good maintenance condition. This was only found on one animal (in the Group IV of male rat after 24 hrs giving of the test preparation), so the incidence was individualized and may be not caused by the test preparation.

Inflammation was a living reaction against to all forms of tissue lesion. The inflammatory process involved the destroying, dissolving, or limiting of the injury-causing agent and the restoration of damaged tissues [17]. The inflammation could be divided into acute and chronic inflammatory. The inflammation was found in some of the test animals, in the liver (4 cases), kidney (1 case), in the intestine (3 cases), and in the stomach (one case). Inflammation that occurred in the test animal categorized as the chronic inflammatory reaction because there was found the lymphocytic infiltration (Fig. 2). This inflammation was induced by a persist stimulation and took a long time for the process. The giving of the test preparation was only in a single dose; it means not caused by the test preparation. The inflammation may be due to bacterial infection. Overall, based on the macroscopic and microscopic observations could be concluded that the one-day oral treatment of the ethanol extract of *S. burahol* leaves did not cause the toxic effects in test animals. In this acute toxicity study, the preparation was only given in a single dose. Therefore, need to be tested in repeated doses to determine the toxic effects of prolonged use.

The results were in-line with the previous study. First, it was found that pseudo-LD_{50} of *S. burahol* ethanol extract was more than 5000 mg/kg b.w. in mice. Second, based on the histopathological observations was found the hydropic degeneration, inflammatory cell infiltration, congestion in the observation of liver organ, but this condition was also found in the control group. On the other hand, hemorrhage and thickening of the alveolar septa were found in kidney and lung, respectively. The changes were also found in the control group, so it was concluded that the histopathological changes were not caused by the *S. burahol* ethanol extract. There was not found any histopathological changes in the other organs of heart, stomach, intestines, and spleen [8]. Thus, based on experiments that have been performed, the ethanol extract of *S. burahol* leaves had pseudo-LD_{50} more than 5000 mg/kg body weight and did not cause toxic effects on organs that were examined both in mice and rats.

CONCLUSION

According to the physical observation, the macroscopic and microscopic investigation could be concluded that the ethanol extract of *S. burahol* leaves did not induce the toxic effect on the body weight, and the organ of male and female SD rats and the toxicity potency were practically non-toxic with the pseudo-LD_{50} >5000 mg/kg body weight.

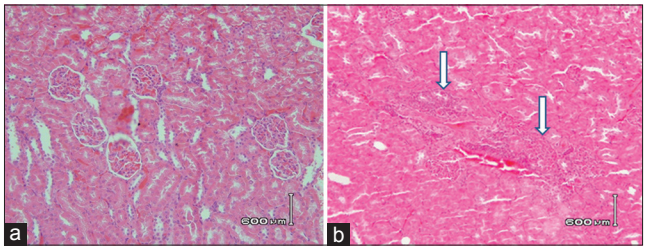

Fig. 2: Preview the kidney histopathology in female rat after 24 hrs of the single oral dose treatment with the ethanol extract of Kepel leaves. (a) Normal kidney in control group, (b) Kidney with lymphocytic infiltration in the inflammatory area was found in Group II (was treated with 200 mg/kg body weight of Kepel extract). Magnification of 10 × 40 and under hematoxylin eosin staining

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