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Short Communication

Acute Toxicity Screening of Katuk Leaf Extract (*Breynia androgyna (L.*) Chakrab. & N.P.Balakr*)* in Mice *(Mus musculus)* Using the Thompson and Weil Methods

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ABSTRACT

Katuk (*Breynia androgyna* (L.) Chakrab. & N.P.Balakr) is a local plant with various medical and food benefits. This plant contains various secondary metabolites, including tannins, polyphenols, alkaloids, saponins, and flavonoids. A toxicity test is intended to determine the safe doses of katuk leaves. This study aimed to conduct acute toxicity testing of katuk leaf extract in mice. The toxicity level was observed from the LD_{50} and observation of potential effects after administering the test doses. The toxicity testing was conducted using the Thompson and Weil method. *Swiss webster* White mice were divided into 5 groups of 5 mice each. The control group was given 1% NaCMC solution, while the other four groups (treatment groups) were given different doses (50, 300, 2000 mg/kg BW) of katuk leaf extract. The results showed that the LD_{50}, calculated using the Thompson and Weil method, was 9954.05 mg/kg BW. The study concludes that the katuk leaf extract is non-toxic and safe in experimental animals at the doses given. However, some behavioural changes (stress and diarrhoea) were observed 24 hours after the treatment at 300 and 2000 mg/kg BW doses.

***Keywords*:** extract, katuk leaves, acute toxicity, mice, Thompson and Weil.

Introduction

Katuk (*Breynia androgyna* L.) Chakrab. & N.P. Balakr) is a species of plant that belongs to the family Euphorbiaceae. Katuk can be grown as an edible hedge or in a garden. According to the United States Department of Agriculture (USDA), this plant can be found in the tropical region of China and tropical countries in Asia, including India, Sri Lanka, Vietnam, Indonesia, Malaysia, Papua New Guinea, and the Philippines. This plant can be used as an ornamental plant and consumed by humans.[1]

In Puspahiang Sub-district, Tasikmalaya Regency, katuk leaves are believed to lower uric acid levels. A study by Fakhrizal [2] also showed that administering 100 g/mL of katuk leaf extract *in vitro* can inhibit the development of uric acid by 59.9%. In addition, an *in vivo* study also showed that katuk leaves had activity against inflammation, pain, and fever.[3]

However, a study revealed that excessive consumption of katuk leaves could cause bronchiolitis. A previous study on the *in vitro* toxicity of methanol leaf extract of katuk from six different places in East Java Province, Indonesia, on human mesenchymal stem cells showed that the extract exhibits low cytotoxicity in cells derived from bone marrow. This was evident by the Inhibition Concentration (LD_{50}) of 2450 mg/L.[4,5,6]

Toxicity testing is crucial to determine any possible adverse effects after the administration of a preparation and to obtain preliminary information that can be used to determine the safe dosage.

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The acute toxicity testing in this study was assessed based on the half-maximal Lethal Dose (LD_{50}) using the Thompson and Weil method. Because this method does not require too many test animals and has a high confidence level.[7]

Therefore, this *in vivo* study evaluated the acute toxicity effect, duration of effect, and the severity and reversibility of katuk (*Breynia androgyna* L.) leaf extract.

Material and Methods

Materials

The study used various materials, including katuk leaves (*Breynia androgyna* L.) Chakrab. & N.P. Balakr) distilled water, chloroform, 10% $FeCl_3$, 1% gelatin, HCl (2N), Mayer's reagent, Dragendorff's reagent, amyl alcohol, Liebermann Burchard reagent, ether, sulfuric acid (2N), vanillin, female Swiss Webster white mice (*Mus musculus*), mouse feed (HI-PRO-VITE 511 Pellet), NaCMC.

Experimental animals

Female Swiss Webster mice weighing 20-30 g were used for the study. They were obtained and maintained in the Animal House Facility of the Department of Pharmacology Perjuangan University. The animals were randomized into experimental and control groups and housed five (5) per group in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Ethical clearance was obtained from BTH University ethics committee on Animal Research (No.057/ec.02/kepk-bth/VI/2022). All experimental protocols complied with the guidelines provided by the committee.

Preparation of samples

The fresh mature leaves of katuk were collected from the Puspahiang district, Tasikmalaya, West Java Province, Indonesia, on March 2022. Plant specimens were authenticated at the School of life sciences and technology, Bandung Institute of Technology, Indonesia. The voucher specimen number was (1158/IT1.C11.2/TA.00/2021).

Preparation of Katuk extract
In making the katuk Simplicia, katuk leaves were first prepared, wet sorted, and rinsed under running water to remove dirt. Afterwards, the leaves were sun-dried to reduce the moisture content. The Simplicial was stored in a place that was free from moisture, sunlight, insects, and other contaminants.[8]

Drying Shrinkage
Based on the Indonesian Herbal Pharmacopoeia, drying shrinkage can be measured in powdered Simplicia with sieve number 8 and a drying temperature of 105°C. The following procedure was used to determine the drying shrinkage. Briefly, 1 to 2 g of Simplicia was weighed into a shallow weighing bottle with a lid, previously heated to the specified temperature, and measured. A flat and uniform sample level in the bottle was achieved by shaking until a layer of approximately 5 to 10 mm thick was obtained. The sample was transferred into the drying chamber at the specified temperature and heated until the weight was constant. Before each reading, the bottle with the lid was left to cool in a desiccator to room temperature.[9,10]

P*hytochemical Screening of Katuk leaves*
The preliminary phytochemical analysis for the identification of alkaloids, phenols, flavonoids, tannins, coumarins, anthraquinones, cardiac glucosides, saponins, and steroids was performed according to the methods described by Awaludin. [11]

Katuk leaf extraction
The powdered plant material was cold macerated with 5 L 70% v/v ethanol with constant shaking for 3 days and then filtered using Whatman filter paper No 1. The filtrate was then concentrated to dryness *in vacuo* at 45°C. The resulting extract was then kept in a desiccator until further use.[12,13]

Oral acute toxicity testing
The test animals, female *Swiss webster* white mice (*Mus musculus*) aged 2-3 months used for this study, were acclimatized for 1 week and weighed 15-30 grams. The mice were fasted for 8 hours before testing and weighed before and after administering the sample. Twenty-five (25) mice were randomly divided into 5 groups of 5 each for this experiment. Group 1, the control group, was given 1% NaCMC while groups 2, 3, and 4 were orally administered 5, 50, 300, and 2000 mg/kg of the plant extract, respectively. The weight of the rats in each group was taken and documented weekly.[14]
Changes in the conditions before and after the treatment were observed. The mice were also weighed every 24 hours post-treatment. The observations were made for 14 days.[14] This method was approved by an ethical committee with a reference number: No.057/ec.02/kepk-bth/VI/2022.

Post treatment observations
After administering the test sample, the experimental animals were observed for signs of toxicity in each test group for 24 hours. Symptoms observed include hanging behaviour, recovery, catalepsy, sedation, tremor, convulsions, flexion, Straub, Haffner, pineal, respiratory, piloerection, salivation, urination, lacrimation, and diarrhoea.[15,16]

Data analysis
Qualitative and quantitative primary data were obtained. The qualitative data were the changes in the mice conditions. The quantitative data were the number of dead mice. The LD_{50} value was determined by counting the number of deaths of animals for 14 days post-treatment with the stipulated doses.[17,18] The Thompson & Weil Method was used to determine the $LD_{50,}$ as shown in the formula below:

$$Logm = LogD + d\,(f + 1) \ldots\ldots (1)$$

Result and Discussion

The katuk leaf Simplicia was made through several stages. The drying shrinkage of the katuk leaf Simplicia was 1.42%. According to MMI, the requirement for drying shrinkage should not exceed 10%. Therefore, the katuk leaf Simplicia from this study met the ministry's requirement. Drying shrinkage >10% may affect the stability and quality of the material because the moisture contained in the sample can lead to microbial growth and enzymatic reactions, which can change the content of the compounds, allowing for changes in pharmacological effects.[19]
The phytochemical screening of the plant sample revealed the presence of tannins, polyphenols, alkaloids, saponins, and flavonoids. These phytochemicals may have been responsible for the various pharmacological effects observed from the use of this plant. Flavonoids, alkaloids, saponins, and tannins have been shown to exhibit vast and potent pharmacological activities such as anticancer, anti-inflammatory, antidiabetic, immune-stimulatory, antioxidant, antimalarial, antimicrobial, and antiviral activities.[20,21] These properties have led to the discovery of novel drug molecules from plants for treating various diseases today. Plants are still the bedrock for the discovery of unique molecules for use in clinical practice. Meanwhile, the presence of these secondary metabolites may have been responsible for the popularity of katuk in primary healthcare usage.
Acute toxicity testing, i.e., determining the LD_{50}, the dose lethal to 50 % of test animals in an experiment, is usually the first step in assessing and evaluating the toxic characteristics of herbal preparation, pharmaceutical leads, etc. It provides safety information on a substance after short-term exposure. Results from acute toxicity studies may serve as a determinant of the therapeutic index of a medicinal product, its dose, and information on its mode of action.[22] In this toxicity study, four doses of the extract were prepared in NaCMC (10%). After administering the test samples, the animals were observed for death and other signs and symptoms of toxicity. Observations revealed that most of the test animals did not experience decreased activities. However, animals in groups P3 and P4 showed signs of toxicity, such as stress, rapid breathing, drowsiness, and diarrheoea (Table 1). The experimental animals' body weights and the number of deaths were determined on day 14th, as shown in Table 2. Initially, there was an increase in body weight in the treatment groups from days 1 to 6. However, on the 7th day, a progressive and gradual weight loss was observed in the animals in groups P2, P3, and P4, possibly due to loss of appetite. Loss of appetite indicates that an animal is sick and can be an early indicator of toxic effects.[23,24]
One of the active compounds in katuk leaves, suspected to be toxic when consumed in high doses, is flavonoids. Flavonoids and tannins are antioxidant compounds with significant pharmacological activities, including immune boosters, anticancer, antiaging, and reduction of oxidative stress.[25-27] However, when antioxidants are in a high concentration, their activities can turn into pro-oxidants, possibly affecting the oxidation rate and causing oxidative stress in cardiac muscle cells due to an imbalance between oxidants and pro-oxidants. In addition, oxidative stress can also cause chronic inflammation in the body.[5] It has been reported (Zhang *et al*.) that excessive consumption of katuk leaves may cause bronchiolitis due to peribronchial inflammation, which may lead to bronchi damage.[5] This study revealed that the LD_{50} of the katuk leaf extract was 9954.05 mg/kg BW, indicating that the plant is safe within the doses used in these experimental animals. However, care should be taken in its consumption, especially in large amounts.

Conclusion

The study showed that katuk leaf extract is a potential source of useful phytoconstituents (alkaloids, tannins, saponins, flavonoids, etc.). It also revealed that Katuk is relatively not toxic at an oral dose of ≥9000 mg/kg body weight in *Swiss webster* white mice.

Conflict of Interest

The authors declare no conflict of interest.

Table 1: Signs of Toxicity in Test Animals 24 Hours after Administration of Preparations

Group	Mice	Signs of Toxicity
P0	1	Did not show any sign of toxicity
(Na CMC 1%)	2	Did not show any sign of toxicity
	3	Did not show any sign of toxicity
	4	Did not show any sign of toxicity
	5	Did not show any sign of toxicity
P1	1	Did not show any sign of toxicity
(5 mg/kgBW)	2	Did not show any sign of toxicity
	3	Did not show any sign of toxicity
	4	Did not show any sign of toxicity
	5	Did not show any sign of toxicity
P2	1	Did not show any sign of toxicity
(50mg/kgBW)	2	Did not show any sign of toxicity
	3	Did not show any sign of toxicity
	4	Did not show any sign of toxicity
	5	Did not show any sign of toxicity
P3	1	The mouse was stressed, marked by tail wagging.
(300mg/kgBW)	2	The mouse was sleepy and experienced rapid breathing.
	3	Did not show any sign of toxicity
	4	Did not show any sign of toxicity
	5	Did not show any sign of toxicity
P4	1	Did not show any sign of toxicity
(2000mg/kgBW)	2	Diarrhea
	3	Diarrhea
	4	Did not show any sign of toxicity
	5	Did not show any sign of toxicity

Table 2: Results of the number of dead test animals at different doses during treatment.

Treatment	Number of Dead Test Animals on Days 1-14													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Group P0 (NaCMC 1%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group P1 (5 mg/kg BW)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group P2 (50 mg/kg BW)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group P3 (300 mg/kg BW)	0	0	0	0	0	0	0	1	1	2	2	3	3	3
Group P4 (2000mg/kg BW)	0	0	0	0	0	0	1	1	2	2	3	3	3	3

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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