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Original Research Article

Subacute Toxicity Test of Ethanol Extract of Sungkai Leaf (*Peronema Canescens* Jack.) on Sgot and Sgpt Levels

Elidahanum Husni*, Dwisari Dillasamola, Miftahul Jannah

Department of Pharmacology, Faculty of Pharmacy, Universitas Andalas Limau Manis, Pauh, Padang City, West Sumatera, Indonesia, 25175

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ABSTRACT

Sungkai plant (*Peronema canescens* Jack.) is an herbal plant that people widely use traditionally to treat various diseases. Sungkai is proven to have immunostimulant, antihyperuricemia, and antibacterial activities. Due to its widespread use and to ensure its safety, it is necessary to evaluate its toxicity. This study aimed to determine the effect of variations in dose and duration of administration of ethanol extract of Sungkai leaves (*Peronema canescens* Jack.) on SGPT and SGOT levels in male white mice. Thirty-six mice divided into four groups (a control group and three treatment groups) of nine each were used for this study. The control group received 0.5% Na CMC suspension, and the treatment groups (II-IV) were given the ethanol extract suspension of Sungkai leaves (*Peronema canescens* Jack.) at a dose of 200, 400 and 800 mg/kg BW p.o, respectively. The extracts were administered daily to the experimental animals for 7, 14, and 21 days. SGPT and SGOT levels were examined on days 8, 15 and 22 using a 5010 v5+ photometer. Data analysis was performed using the SPPS with a two-way ANOVA test based on variations in dose and duration of administration. The results of this study indicate that the ethanol extract of Sungkai leaves has no significant effect on SGPT and SGOT levels (p>0.05) based on variations in the dose and duration of administration.

Keywords: *Peronema canescens* Jack, SGOT, SGPT, Sungkai, Subacute toxicity .

Introduction

Natural products of plant origin have been shown to function as immunomodulators, and this has been explored in the management of certain diseases, including oxidative stress, cancers, HIV, inflammations, diabetes, etc. One plant widely used as a natural immune booster is the sungkai plant (*Peronema canescens* Jack.). Sungkai (*Peronema canescens* Jack.) is one of the leading export commodities specifically for Sumatra and Kalimantan.[1,2]

Phytochemical screening of Sungkai revealed the presence of various secondary metabolites, including alkaloids, steroids, terpenoids, tannins, and saponins.[3] The plant has also been shown to possess immunostimulant effects by increasing the phagocytic capacity of macrophages, decreasing segmental neutrophil cells, leukocyte counts, percentage of lymphocyte cells, and increasing cytokines.[4] Another study also highlighted the antihyperuricemia activity of sungkai extracts through the reduction of blood uric acid levels in mice.[5] The extract was also reported to exhibit antibacterial activity against the growth of *E. coli*.[6] During the COVID-19 pandemic, many people chose herbal medicines to maintain health. One herb widely consumed as a decoction to boost immunity was sungkai leaves. The antioxidant and antibacterial compounds contained in the leaves have been regarded as natural immune modulators to boost the immune system.[7]

The use of traditional medicine predates formal health services, and nowadays, herbs are used as a complement to orthodox treatment.[8]

*Corresponding author. E mail: elidahanumhusni@phar.unand.ac.id
Tel: +62 813-6398-9864

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In the last decade, many have turned to traditional medicine products and practices with the assumption that 'natural means safe', which is not necessarily true. All effective drugs can have adverse reactions, including herbal medicines. For this reason, in using herbal medicines, it is important to consider the dose, time of use, method of use, and selection of drugs for the disease.[9,10,11] Although often referred to as safe, it has been shown that many medicinal plants have the intrinsic potential to be toxic and have been implicated in herb-herb or herb-drug interactions.[12] Meanwhile, there are several cases of adverse reactions to the use of herbal medicines that prove that herbal medicines are not entirely safe.

Toxic substances of herbal or chemical origin can affect and damage vital organs in the body, especially the liver, which is susceptible to chemical attacks due to its involvement in the biotransformation of chemicals in the body. In the biotransformation process of food contaminated with toxins, toxic reactive chemicals can be formed, exposing the liver to frequent assault by toxic metabolites of these chemicals.[15]

In a retrospective study in Korea, there were cases of liver damage due to herbal induction in 27 out of 4,769 patients (0.6%) with musculoskeletal disorders who received traditional Chinese medicine (TCM). Herbal medicine has been reported to contribute to 24.2% of cases of liver damage by drugs in China, 11% in Spain, and 9% in the United States. [15] These case reports point to the importance of safety and toxicity testing of herbal medicines and traditional medicine products. This effort is one of the important steps in the strategy to improve the development of traditional medicine.

The dose and duration of administration of a drug substance are some important factors that determine its safety and toxicity because toxicity is a function of exposure, dose, and time.[16] With increasing numbers of herbs-induced toxicity and limited scientific reports on the safety and toxicity of sungkai leaves, there is a need to investigate its toxicity using experimental animals. Therefore, this study aims to evaluate the effect of sungkai leaf extract on SGPT and SGOT blood levels as toxicity indicators with respect to dose using experimental animals in subacute treatment.

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Material and Methods

Animals

Thirty-six healthy male white mice aged 2-3 months with body weights of 20-30 g were used in this study. The animals were kept in polypropylene cages in the animal house facility of the institute maintained at room temperature (22℃). They were given rodent pellets and had access to sufficient food and water *ad libitum* and a 12-hour light/dark cycle. The animals were cared for according to the recommendations for the use and care of laboratory animals provided by the Faculty of Pharmacy Ethics Committee of Universitas Andalas. Before treatment, the test animals were acclimatised for 7 days, during which they were also observed for changes in appetite and body weights.

Ethical Approval

Ethical approval for the use of the animals for this study was obtained from the Faculty of Pharmacy Ethics Committee of Universitas Andalas, with approval number 13/UN.16.10.D.KEP-FF/2023.

Plant Materials and Collection

Samples of sungkai (*Peronema canescens* Jack.) were obtained in June 2022 from *UPTD Rumah Potong Hewan Aia Pacah* Padang City, West Sumatera. The plant sample was identified and validated by Dr Nurainas of Andalas University Herbarium (ANDA), Department of Biology, Faculty of Mathematics and Natural Science, Universitas Andalas, Padang City, West Sumatera. A voucher specimen number 260/K-ID/ANDA/V/2022 was assigned.

Preparation of the Extract

About 4 kg of the fresh plant sample was dried and ground to a fine powder (700 g). This sample was subsequently macerated with 70% ethanol at a sample-to-solvent ratio of 1:10 in a dark-coloured glass container for 6 hours. The filtrate was dried using a rotary evaporator at reduced pressure.

Phytochemical Screening

Qualitative chemical testing was carried out on the extract to determine its phytochemical components (e.g. alkaloids, saponins, phenols, flavonoids, steroids, and terpenoids) using standard protocols.[9]

Administration of Sungkai Leaf Ethanol Extract Suspension

The plant extract at concentrations of 200, 400 and 800 mg/kg bw p.o, respectively, was administered daily to the experimental animals for 7, 14, and 21 days. The control group received 0.5% Na CMC. Blood samples were collected from the orbital sinus of the eye on days 8, 15, and 22 for examination of SGPT and SGOT levels in the experimental animals.

Statistical analysis

The data were analysed by two-way ANOVA between the time (duration of administration) and doses. Subsequently, the significant results were analysed by Duncan's multiple range test (p<0.05) using IBM SPSS Statistics V24.

Results and Discussion

Phytochemical screening was conducted to examine secondary metabolites contained in sungkai leaves. The results obtained showed that the extract of sungkai leaves contained alkaloids, flavonoids, phenolics, saponins and triterpenoids (Table 1).

Thirty-six albino mice (male) weighing 20-30 grams were used for this study. They were selected due to their ability to provide more stable research results because, apart from having a faster metabolic activity, their biophysical condition is not affected by menstrual cycle and pregnancy. Similarly, the dosing of the animals was selected based on the usual dose of sungkai leaf extract that provides immunostimulant effects, as previously reported.[4] The ethanol extract of sungkai leaves was solubilised in 0.5% Na CMC, which was also used as a negative control vehicle.[17]

Table 1: Results of Phytochemical Screening

Phytochemical screening	Result
Alkaloids	+
Flavonoid	+
Phenolic	+
Saponin	+
Steroid	+
Terpenoid	+

(+) = contains a secondary metabolite
(–) = does not contain a secondary metabolite

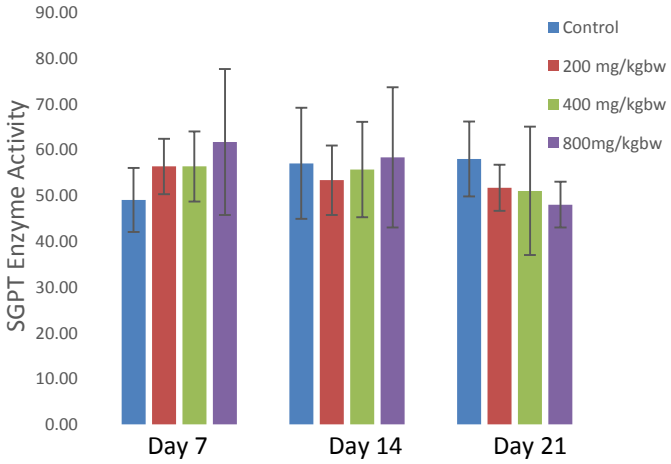

Figure 1: Effect of Sungkai Leaf Ethanol Extract Dosage and Duration of Administration on SGPT Levels

Subacute toxicity test is conducted to ensure the *in vivo* safety of traditional medicinal plants when administered over some time. The subacute toxicity test aims to provide information on the toxicity of a substance that is not detected in acute toxicity studies, to establish the dose(s) that does not cause toxic effects and to provide information on the cumulative impact of a test substance on target organs after a certain period of administration. In this study, oral doses of the test preparation were given daily for 7 days, 14 days, and 21 days (Table 2).

After the scheduled treatments, blood samples from the different experimental groups were collected from the orbital sinus of the eye and centrifuged for 10 minutes at 3000 rpm. The clear supernatant layer (serum) was collected into a microtube for further evaluation using a micropipette. The serum was examined for SGPT and SGOT levels. SGPT and SGOT are enzymes which are biomarkers for liver cell abnormalities or injuries. During liver damage, changes in membrane permeability occur, resulting in enzymes such as SGOT, SGPT, lactate dehydrogenase, gamma-glutamyl transaminase, and arginase leaving the cell freely into blood vessels, leading to their increase in blood levels. Among all these enzymes, the key indicators for liver damage are SGOT and SGPT because their increase is more drastic and with rapid onset.[18]

The examination of SGPT and SGOT levels was carried out using a 5010 v5+ photometer. The principle of the SGPT enzymatic method is that Serum Glutamate Pyruvate Transaminase (SGPT) catalyses transaminase from L-Alanine and 2-oxoglutarate to form L-Glutamate and pyruvate. Then, the pyruvate formed is reduced by Lactic Dehydrogenase (LDH) to D-Lactate, and the Nicotinamide Adenine Dinucleotide (NADH) is oxidised to NAD. Also, the principle of the SGOT enzymatic method is the catalysis of transaminase from L-Aspartate and 2-oxoglutarate to form L-Glutamate and oxaloacetate by Serum Glutamate Oxaloacetate Transaminase (SGOT). Oxaloacetate is then reduced by Malate Dehydrogenase (MDH) to L-Malate, followed by the oxidation of Nicotinamide Adenine Dinucleotide

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(NADH) to NAD. The amount of NAD oxidised is directly proportional to the levels of SGPT and SGOT contained in the test serum, so the photometer provides absorbance results for the calculation of the levels of SGPT and SGOT in the serum of treated animals.[19]

Statistical results showed that there were no significant differences in the mean SGPT levels (p>0.05) between the control group and experimental groups (200, 400, and 800 mg/kg bw), respectively. This relationship was also observed with respect to the duration of administration of the extracts, which shows that the average SGPT levels between the control group and the test groups after 7, 14, and 21 days of administration were not significantly altered (p>0.05), (Table 3 and Figure 1).

The range of SGPT levels obtained was 41-75 U/L. The average SGPT levels of the control and treatment groups at extract doses of 200 mg/kg bw, 400 mg/kg bw, and 800 mg/kg bw were 54.67±9.10 U/L, 53.78±6.21 U/L, 54.33±10.68 U/L, and 56.00±12.09 U/L, respectively. While the average SGPT levels based on the duration of administration on days 7, 14, and 21 were 55.83±9.15 U/L, 56.08±11.35 U/L, and 52.17±8.05 U/L (Table 3).

Results of the two-way ANOVA analysis of SGOT levels show that the average blood SGOT levels of mice do not have a significant effect by dose variation, duration of administration, and interaction between dose and duration of administration (p>0.05). The average levels of SGOT in the control group against the treatment group at test concentrations 200 mg/kg bw, 400 mg/kg bw, and 800 mg/kg bw did not show significant differences (p>0.05). It also showed that the average SGOT levels between the control group and the test groups for 7 days, 14 days, and 21 days were not significantly different (p>0.05) (Table 4, Figure 2).

Table 2: Grouping of experimental animals

	7 days	**14 days**	**21 days**
Control	3 mice were given 0.5% Na CMC suspension.	3 mice were given 0.5% Na CMC suspension.	3 mice were given 0.5% Na CMC suspension.
Dose 200mg/kgbw	3 mice were given a suspension of ethanol extract of sungkai leaves at a dose of 200 mg / kgbw.	3 mice were given a suspension of ethanol extract of sungkai leaves at a dose of 200 mg / kgbw.	3 mice were given a suspension of ethanol extract of sungkai leaves at a dose of 200 mg / kgbw.
Dose 400mg/kgbw	3 mice were given a suspension of ethanol extract of sungkai leaves at a dose of 400 mg / kgbw.	3 mice were given a suspension of ethanol extract of sungkai leaves at a dose of 400 mg / kgbw.	3 mice were given a suspension of ethanol extract of sungkai leaves at a dose of 400 mg / kgbw.
Dose 800mg/kgbw	3 mice were given a suspension of ethanol extract of sungkai leaves at a dose of 800 mg / kgbw.	3 mice were given a suspension of ethanol extract of sungkai leaves at a dose of 800 mg / kgbw.	3 mice were given a suspension of ethanol extract of sungkai leaves at a dose of 800 mg / kgbw.

Table 3: Effect of Sungkai Leaf Ethanol Extract Dosage and Duration of Administration on SGPT Levels

Dose	**Average ± SD**			**Average ± SD**
	7	**14**	**21**	
Control	49.00 ± 7.00	57.00 ± 12.12	58.00 ± 8.19	54.67 ± 9.10
200 mg/kgbw	56.33 ± 6.03	53.33 ± 7.57	51.67 ± 5.03	53.78 ± 6.21
400 mg/kgbw	56.33 ± 7.64	55.67 ± 10.41	51.00 ± 14.00	54.33 ± 10.68
800 mg/kgbw	61.67 ± 15.95	58.33 ± 15.31	48.00 ± 5.00	56.00 ± 12.09
Average ± SD	55.83 ± 9.15	56.08 ± 11.35	52.17 ± 8.05	

Table 4: Effect of Dose of Sungkai Leaf Ethanol Extract and Duration of Administration on Average SGOT levels

Dose	**Average ± SD**			**Average ± SD**
	7	**14**	**21**	
Control	89.67 ± 11.72	101.67 ± 11.59	100.33 ± 6.81	97.22 ± 10.04
200 mg/kgbw	101.00 ± 8.54	107.00 ± 6.00	101.33 ± 13.58	103.11 ± 9.37
400 mg/kgbw	99.67 ± 13.50	107.00 ± 2.00	96.00 ± 6.56	100.89 ± 7.35
800 mg/kgbw	89.33 ± 4.93	89.67 ± 8.08	97.00 ± 16.09	92.00 ± 9.70
Average ± SD	94.92 ± 9.67	101.33 ± 6.92	98.67 ± 10.76	

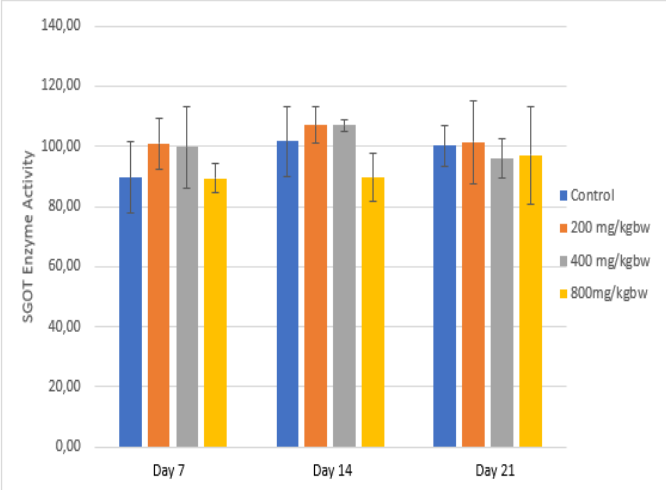

Figure 2: Effect of Sungkai Leaf Ethanol Extract Dosage and Duration of Administration on SGPT Levels

The range of SGOT levels obtained is 81-117 U/L. The average SGOT levels of each control group and treatment group given the extract at a dose of 200 mg/kg bw, 400 mg/kg bw, and 800 mg/kg bw were 97.22±10.04 U/L, 103.11±9.37 U/L, 100.89±7.35 U/L, and 92.00±9.70 U/L, respectively. While the average SGPT levels on days 7, 14, and 21 were 94.92±9.67 U/L, 101.33±6.92 U/L, and 98.67±10.76 U/L (Tables 4 and 3).

The average SGOT levels obtained fluctuated in the treatment groups each week. Based on the literature, SGOT enzyme is not specific in assessing liver function health. SGOT enzyme is not only found in the liver but also the heart muscle, lungs, skeletal muscle, pancreas, and kidneys. So, the levels of SGOT contained in the blood are not specific indicators of the condition of the liver because this enzyme can also be contributed by other organs or tissues in the body.[19]

Overall, SGPT and SGOT activities in all treatment groups were in the normal range, and the results of the two-way ANOVA test also did not show any significant differences (p>0.05). According to Charles River's Standard, the normal value of SGPT in mice is 41-131 U/L, while that of SGOT is 55-352. These results indicate that the treatment with ethanol extract of sungkai leaves (*Peronema canescens* Jack.) at doses of 200 mg/kg bw, 400 mg/kg bw, and 800 mg/kg bw compared to the control group in the 21-day administration range did not have a significant effect on SGPT and SGOT enzyme activity in mice. So it can be concluded that the ethanol extract of sungkai leaves (*Peronema canescens* Jack.) may not significantly affect blood levels of SGPT and SGOT and hence considered safe after oral administration in the doses stipulated and may not induce subacute toxicity in mice.[20]

Conclusion

The results of this study showed no significant effect of dose variation and duration of administration of ethanol extract of sungkai leaves (*Peronema canescens* Jack.) on SGOT and SGPT levels in the treated animals, and the leaf extract of sungkai may be safe following oral administration.

Conflict of Interest

The authors declare no conflict of interest.

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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