



RESEARCH ARTICLE

Effect from Repeated Dose of Ethanol Extract of Singawalang Leaves (*Petiveria alliacea*. L) on Histopatologic Findings of Liver and Kidney with Aminotransferase Enzymes and Creatinine levels of Male Mice

Mulyani Yani*, Sukmawati Ika K, Parikesit Thosa S

Laboratory of Pharmacology, Department of Pharmacology, Bandung School of Pharmacy, Bandung, Indonesia.

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ABSTRACT

The safety and efficacy of herbal products have been guaranteed usually based on their long history of clinical application one of them is singawalang (*Petiveria alliacea* .L). However, increasing concern exists regarding the lack of scientific evidence for the safety and efficacy of herbal medicines. To investigate the toxicity effects from repeated dose of ethanol extract of singawalang leaves on liver and kidney, the research was carried out based on method from OECD Guideline: Repeated Dose 28-Day Oral Toxicity Study in Rodents. The ethanol extract of singawalang leaves was orally administered daily to three group of tested mice at dose of 1000, 3000, and 5000 mg/kg bw/day for a period of 28 days also one group have been used as control group (NaCMC 0,5%). The study was carried out to determined percentage of necrosis-liver cell, diameter of glomerular kidney, ALT, AST, and creatinine serum levels. The data obtained were analyzed statistically using One Way ANOVA method ($p < 0,5$). The ethanol extract of singawalang leaves show significant effect of toxicity at dose of 3000 and 5000 mg/kg bw by increased percentage of necrosis-liver cell, ALT, AST, and creatinine serum levels compared to control group. The data also shows at dose of 1000 mg/kg bw there is no toxicity effect was observed.

KEYWORDS

Toxicity, Singawalang Leaves (*Petiveria Alliacea*), Liver and Kidney, Aminotransferase, Creatinine, Male Mice

INTRODUCTION

Along with the attention paid to the safety of herbal medicines, the number of toxicity studies of herbal medicines is increasing. However, the number of toxicity studies is lacking, and several have presented controversial data.

In Korea, for example, one study indicated herbal medicines as the major cause of drug-induced liver disease; however, another prospective study reported clinical data that suggested the safety of herbal medicines .

Singawalang (*Petiveria alliacea* .L) is a perennial herb widely used in folk medicine in the Caribbean, Central and South America. Leaf infusions or root powder are known for their anti-spasmodic, anti-rheumatic, anti-inflammatory, nociception, hypoglycemic and abortifacient properties. Additionally, aqueous infusions have been used in leukemia and breast cancer treatment.

***Address for Correspondence:**

Mulyani Yani,
Laboratory of Pharmacology,
Department of Pharmacology,
Bandung School of Pharmacy,
Bandung, Indonesia.
E mail ID: yani.mulyani@stfb.ac.id

To clarify the toxic effects of singawalang, the epidemiology of its toxicity and risk factors should be scientifically investigated. The pharmacological and toxicological processes are usually based on animal studies and clinical evaluation. Animal toxicity studies are important for predicting side effects and deciding the safe dose of drugs before clinical studies; thus, animal toxicity studies are the “gold standard” for toxicity assessment.

Organization for Economic Cooperation and Development (OECD) guidelines issued specific guidelines for preclinical sub chronic toxicological evaluations (OECD, 2008). Therefore, toxicological evaluations of ethanol extract of singawalang leaves in laboratory animals is needed to provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. Therefore, the 28-days oral toxicity of ethanol extract of singawalang leaves were evaluated using OECD Guidelines No. 407 (OECD, 1998).

MATERIAL & METHODS

Animals

Albino male mice (21-30 g, 8-12 weeks) were used for the study. They were housed in cages at a temperature of 25 ± 1 °C with 12h fluorescent light and 12h dark cycle in an animal house facility. The mice had free access to water ad libitum throughout the study duration except during actual measurements. All experiments were carried out between 09:00 and 17:00 h. The Health Research Ethics Committee Faculty of Medicine Universitas Padjadjaran Bandung, approved the experimental protocol (525/UN6.C.10/PN/2017).

Plant Extraction

Plant material was collected at Manoko, Lembang, Bandung on Januari 2017. The plant material was identified by Jatinangor Herbarium, Laboratory of Plant Taxonomy, Department of Biology FMIPA UNPAD. Dry ground leaves and stems (2 kg) from singawalang were extracted using reflux

extraction method with 96% ethanol. The extract was filtered and evaporated until half-volume.

Repeated Dose 28-day Oral Toxicity Study of Ethanol Extract of Singawalang Leaves in Mice

The subacute oral toxicity study was performed according to the OECD Guideline 407 “Repeated Dose 28-day Oral Toxicity Study in Rodents” (OECD, 1998). There were four groups of albino mice (5 mice/sex/group) viz. Vehicle (Na CMC 0,5%, double distilled water), ethanol extract of singawalang leaves (1000, 3000, 5000 mg/kg). Vehicle or ethanol extract of singawalang leaves was administered daily for 28 days.

Blood Collection and Biochemical Analysis

At the end of the experiment, the mice were euthanatized under chloroform anesthesia. Blood was collected and organs (kidneys and liver) were isolated and weighted. Blood samples used to analysis the levels of alanine transferase, aspartate transferase, and serum creatinine using specific kits and measurement with spectrophotometer. Organ samples (kidneys and liver) from one mice were fixed in 10% formalin for histopathological examination.

Statistical Analysis

The data were represented as mean \pm standard error of mean (SEM). Data analysis was performed using PASW Statistics 18 software. Data were analyzed using one-way ANOVA followed by Tukey test for each parameter separately. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Histopathological Findings of Liver and Kidneys Organ

Ethanol extract of singawalang leaves at dose of 3000 and 5000 mg/kg caused liver cellular damage, which was evident from percentage of cell necrosis with value of 11.56% and 39.31%, respectively. Histopathological analysis of kidney also from the same dose showed

Table 1: Percentage of liver cell necrosis and diameter of glomerular kidney

| Parameter | VC | Ethanol extract of singawalang leaves (mg/kg, oral) | | |
|-------------------------------|--------------|-----------------------------------------------------|--------------|--------------|
| | | 1000 | 3000 | 5000 |
| % liver cell necrosis | 1.14 | 7.12 | 11.56 | 39.31 |
| Diameter of glomerular kidney | 68.73 ± 1.75 | 69.75 ± 4.30 | 60.73 ± 2.08 | 59.32 ± 3.04 |

Table 2: Data of blood biochemistry

| Parameter | VC | Ethanol extract of singawalang leaves (mg/kg, oral) | | |
|--------------------|--------------|-----------------------------------------------------|--------------|--------------|
| | | 1000 | 3000 | 5000 |
| ALT (U/L) | 11.39 ± 1.29 | 11.32 ± 1.31 | 22.05 ± 1.79 | 40.70 ± 3.89 |
| AST (U/L) | 48.65 ± 3.83 | 47.33 ± 3.98 | 67.19 ± 4.77 | 80.85 ± 5.57 |
| Creatinine (mg/dL) | 0.23 ± 0.025 | 0.23 ± 0.027 | 0.23 ± 0.034 | 0.28 ± 0.027 |

shrinkage diameter of glomerular kidneys compared to vehicle group.

Blood Biochemistry

There was a significant increase ($p < 0.05$) in alanine transferase, aspartate transferase, and creatinin level of ethanol extract of singawalang leaves at dose of 3000 and 5000 mg/kg compared to vehicle group.

DISCUSSION

There were significant alterations in liver and kidney function reflected by the results of the present study where increased in ALT, AST and creatinine after sub chronic oral administration of ethanol extract of singawalang leaves (3000 and 5000 mg/kg) but not at lower doses. Liver is the primary target organ of toxicity because as it's the first organ exposed to the metabolite

that is absorbed in the small intestine. AST and ALT be the two aminotransferase enzymes present in abundant concentration in the liver which represent liver function, whereas creatinine is a good indicator of kidney function and alteration in their levels reflects the hepatic and renal toxicity, respectively. Administration of ethanol extract of singawalang leaves (3000 and 5000 mg/kg) for 28 days caused a significant elevation in the ALT, AST and creatinine level thus indicates hepatic and renal adverse effects. Increase in percentage of cell necrosis of the liver and shrinkage of glomerular kidney after administration of ethanol extract of singawalang leaves in the 28 days repeated dose toxicity study during necropsy of mice supports the results.

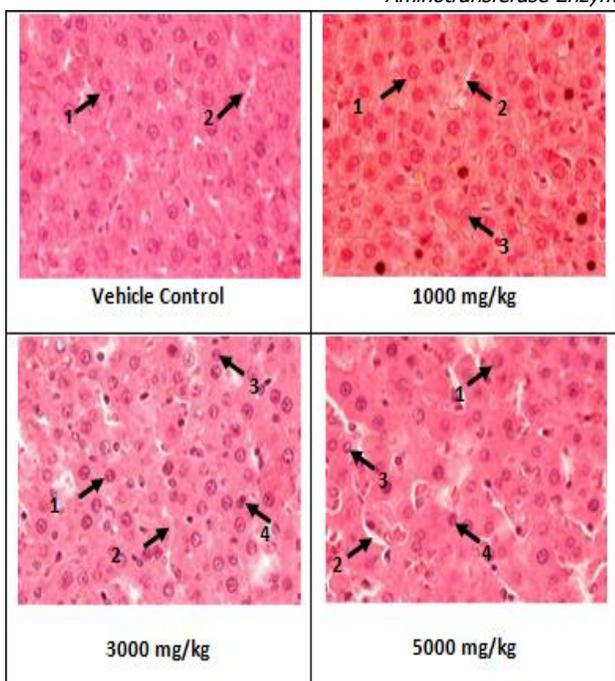


Figure 1. Effect of repeated 28-day dose of ethanol extract of singawalang leaves on histopathological findings of liver at 400x zoom

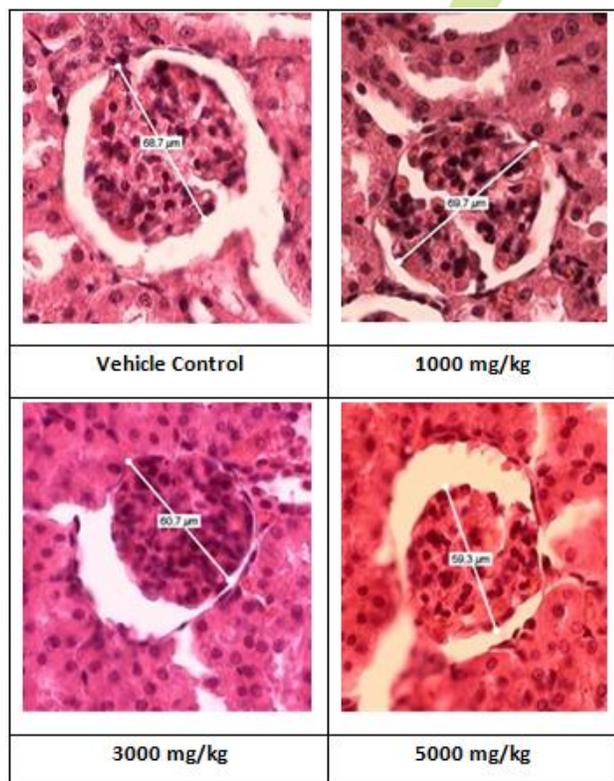


Figure 2: Effect of repeated 28-day dose of ethanol extract of singawalang leaves on histopathological findings of kidneys at 200x zoom

CONCLUSIONS

In conclusions, the 28 days repeated dose of ethanol extract of singawalang leaves showed significant toxicological effect on liver and kidneys at dose of 3000 and 5000 mg/kg. However at dose of 1000 mg/kg there was no toxicological effect have been observed.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose

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