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SUBCHRONIC ORAL TOXICITY TEST FOR 28 DAYS OF SAUROPUS ANDROGYNOUS L. MERR. LEAVES SOUP IN WISTAR FEMALE RATS

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ABSTRACT

Objective: The objective of the study was to evaluate the subchronic toxicity of Sauropus androgynus L. Merr. leaves soup.

Methods: Subchronic oral toxicity tests were carried out for 28 days in female Wistar rats using conventional methods. Thirty rats were divided into six groups, namely, one control group and three test groups with each extract being given at a dose of 500 mg/kg body weight (BW), 1000 mg/kg BW, and 3000 mg/kg BW, and one group satellite control and satellite test group with doses of 3000 mg/kg BW were carried out for 14 days after 28 days of treatment to see the effects of reversibility. All rat groups were observed for behavior, development of BW, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), creatinine serum, ratio of liver and lung organs to BW, and histology of liver and lung.

Results: The macroscopic observation of rat's lung and rat weight ratio did not show a significant difference to the control group (p>0.05). In addition, the ratio of liver volume to BW was significantly different between the doses of 1000 mg/kg BW and 3000 mg/kg BW with satellite groups 3000 mg/kg BW (p<0.05). Levels of SGOT and SGPT as well as liver and lung histopathology scores did not show a significant difference to the control group (p>0.05). However, creatinine serum had the highest increase at a dose of 500 mg/kg BW and a dose of 1000 mg/kg BW. Reversibility effects were not seen after 14 days of the past day given *Sauropus androgynous* soup for 28 days in female Wistar rats.

Conclusion: The given of *S. androgynous* soup for 28 days for female Wistar rats did not show any specific toxicity effect so that its use was relatively safe for the consumption under 30 days. This study is expected to be the information source about the safety profile of *S. androgynus* leaves soup consumption.

Keywords: Sauropus androgynous, Subchronic toxicity, Female Wistar rats.

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INTRODUCTION

Breast milk is the best food for newborns, given when babies are 0-6 months old. The nutrition in breast milk is very important for the growth and development of the baby. The survey results stated that 38% of mothers stopped to breastfeed their babies due to insufficient production. Therefore, a solution is needed to be able to increase the quantity of breast milk production. One type of herbal plant that has proven its scientific test to increase breast milk production is Sauropus androgynus [1]. In Indonesia, S. androgynus is called and known as Katuk. In general, people in Indonesia consume the leaves of this plant to be made as vegetables in the form of soup or stew. Some studies showed that the use of S. androavnus leaves can increase the production of breast milk up to 50.47% without reducing the quality of breast milk [2,3]. Apart from being galactagogue, Sauropus androgynous leaves also function as antibacterial, antioxidant, and anti-inflammatory [4]. In addition, this plant can also lose weight and heal wounds [5,6]. A study conducted by Warditiani et al. also show that the saponin content in katuk leaves also has antidislipidemia activity and can be used to prevent cardiovascular disorders [7].

However, several studies have suggested the emergence of side effects in the overuse of *S. androgynous* leaves such as constipation, drowsiness, and respiratory problems such as bronchiolitis obliterans. Papaverine which is one of the alkaloids contained in *S. androgynus* is considered as potential cause of bronchiolitis obliterans. However, in some research findings, these side effects appear more often if these plants are consumed in the form of juice [8].

Therefore, an evaluation was needed to identify the toxic effects that may arise in the long-term use of *S. androgynus* leaves soup because

even though it was considered safe, other possible unexpected toxicity effects on the body due to prolonged use were not known yet.

MATERIALS AND METHODS

Plant material used

The plants used in this study were the leaves of *S. androgynus* obtained from Toga Garden of Ubaya Training Center in Trawas, East Java, and were determined by the University of Surabaya PIPOT (Traditional Medicine Information and Development Center). Other materials used were carboxymethyl cellulose (CMC) Na as suspending agent and aquadest as solvents, 0.9% NaCl solution, and 10% buffered formalin.

Animal experiment

The animals used in this experiment were 30 female Wistar rats aged 6–8 weeks, with variants in weight not more than 20%. All rats were placed in cages with a room temperature of $22\pm3^{\circ}\text{C}$ and humidity of 30--70%, with 12 h of bright lighting and 12 h of darkness. Rats were adapted for the first 7 days before the study and each rat was weighed to ascertain if the weights met the research requirements. They were kept in cages made of waterproof, strong, and easy to clean material, and the maintenance room was free of noise. According to the Cage Space Guidelines for Animals Used in Biomedical Research (2008), the cage area for rats weighing 100--200 g is the cage area of 148.4 cm^2 and height 17.8 cm.

Soup preparation

S. androgynous leaves were randomly picked from top, middle, and bottom of the stem. Leaves were cleaned with running water to remove dirt and dried at room temperature without heating so as

not to damage the metabolites. The water contained in *S. androgynus* leaves was removed by freeze-drying to let the leaves dried. After that, *S. androgynus* leaves were crushed using a mortar and stamper until fine powder was formed and the powder was sieved using 100 Mesh. Then, the moisture content of the powder was measured by moisture content balance. If the *S. androgynus* leaves powder was not ready for use yet, it could be stored in the freezer in the degree of -20° C.

Mucilage was made by sprinkling CMC Na powder to the surface of the water for 20 times the weight of CMC Na then left for about 5 min until all mucilages expanded. After all CMC Na expanded, the mucilage was stirred until homogeneous with the mortar and stamper. *S. androgynus* leaves were weighed according to the dose, put in a beaker glass then added by water, stirred until homogeneous, covered with aluminum foil, and simmered for 5 min. *S. androgynus* leaves were inserted into mucilage after *S. androgynus* were cooled, stirred until homogeneous, transferred to the container, and added by water until certain volume.

28 days - subchronic toxicity test

Rats were divided into six groups, in which one group as a negative control group, three groups were the test group with a dose of 500 mg/kg body weight (BW), 1000 mg/kg BW, and 3000 mg/kg BW, and the last two groups were satellite groups used for observation of the toxicity reversibility effects after the 14 days after the administration of the test preparation. Two satellite groups were high-dose groups and satellite control groups. Soup was given every day for 28 days. Experimental animals were fasting from any food for overnight (18 h) before administration of the test material. Observation of the toxic symptoms and clinical symptoms in the form of changes in skin, fur, eye clarity, changes in the way of walking, strange behavior (e.g., walking backward), seizures, etc., was carried out every day for 28 days. For the satellite group, the experiment was continued for 14 days afterward to detect the recovery process from toxic effects. Observation of liver and kidney function on experimental animals was carried out by collecting blood plasma before sacrificed on day 29 and satellite group on day 43. Quantitative determination was done by calculating blood biochemical levels using a spectrophotometer which included serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and creatinine serum. After that, the liver and lung were taken to be measured for the weight and relative volume, also went to microscopic examination through histopathological examination.

Statistical analysis

The data were obtained statistically from one-way ANOVA method.

RESULTS AND DISCUSSION

Oral subchronic toxicity test for 28 days is a test to detect toxic effects that arise after the administration of the test dosage with repeated doses administered orally to test animals for a portion of the animal's age, but not more than 10% of the entire animal's age. The purpose of this test is to obtain information on the toxic effects of substances that are not detected in acute toxicity tests, information on the possibility of toxic effects after repeated exposure to the test product, dosage information that does not cause toxic effects, and study the cumulative and reversible effects of these substances [9].

After giving *S. androgynus* leaves soup for 28 days, there was no sign of physical abnormality in all treatment groups such as no hair loss, the eyes were still clear, no nasal mucus, and no diarrhea. Therefore, it could be concluded that experiment animals were healthy during the research. The weight profile on subchronic toxicity test of control and experimental groups is shown in Fig. 1. There was an increase on the weight of rats which were given *S. androgynus* leaves soup, which indicated that the soup did not give general toxic effect and did not affect their appetite.

In this research, all SGOT enzyme values in all treatment groups were in normal range of 65-203 U/L for female rats [10]. As shown in Table 1, the

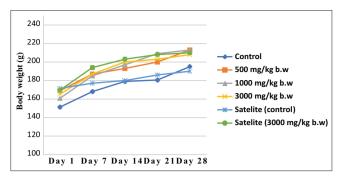


Fig. 1: Profile of weight increase in female Wistar rats after being given *S. androgynus* leaves soup for 28 days

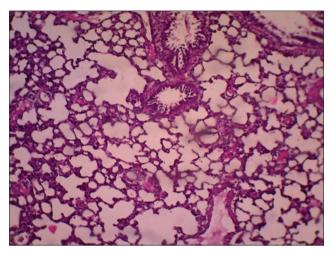


Fig. 2: Micrograph photo of liver tissue in female Wistar rats after given control for 28 days

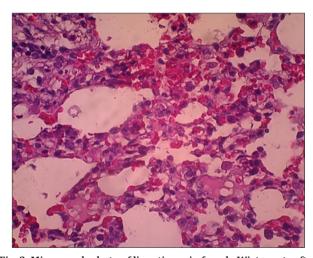


Fig. 3: Micrograph photo of liver tissue in female Wistar rats after given *S. androgynus* leaves soup for 28 days

highest average values of SGOT enzyme was detected on satellite group of $3000 \, \text{mg/kg}$ BW with $121.2 \, \text{U/L}$ and the lowest average SGOT enzyme level was in the group with the dose of $1000 \, \text{mg/kg}$ BW. SGOT enzyme is located on body organ's cells, where the highest number located in cardiac muscle, then in liver, kidneys, and pancreas. SGOT enzyme increases on the patients of myocardial infarction, after exercising, and under the effect of some medicine or chemical compounds [10].

In the results of this study, all SGPT enzyme value in all treatment groups also in a normal range of 16-48~U/L for female rats [11]. The highest

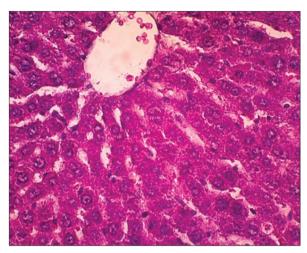


Fig. 4: Micrograph photo of lung tissue in female Wistar rats after given control for 28 days

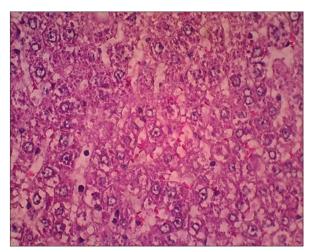


Fig. 5: Micrograph photo of lung tissue in female Wistar rats after given Sauropus androgynus leaves soup for 28 days

average SGPT values were in a group with the dose of 3000 mg/kg BW with 45.8 U/L, and the satellite group with 43 U/L was group with the lowest SPGT values. Based on the statistical analysis, there was no meaningful difference between treatment groups regarding the SGOT and SPGT. The SPGT enzyme is mostly located on body tissue cells and the main source in liver cells, while the less number was located in cardiac and skeletal muscle compared to SGOT. SPGT enzyme increases in liver damage and disorder and from the influence of some drugs and chemical compound so that SPGT enzyme is used as the most specific indicator of liver damage [12].

Giknis and Clifford stated that the normal level of creatinine in rats is 0.2–0.6 mg/dl [11]. The average of creatinine serum in each treatment group showed a higher value than normal value both for tested groups and control groups. The tested groups of 500 and 1000 had the highest creatinine value with 1.18 mg/dl and 1.19 mg/dl, respectively. From ANOVA analysis, there was a meaningful difference among the tested groups of 500 mg/kg BW and 1000 mg/kg BW with control satellite groups and satellite group of 3000. However, this could not be indicated that *S. androgynus* leaves soup caused subchronic toxicity since it had to be supported by kidney's histopathologist data. Besides that, the increase in creatinine serum is not only caused by kidney damage but also other influencing factors such as BW, muscle mass, race, age, gender, muscle metabolism, and hydrate status [13].

Table 1: Profile of liver and kidney biochemistry parameter

Groups	Biochemical parameters				
	SGOT (U/L)	SGPT (U/L)	Creatinine (mg/dl)		
Control	106.60±9.76	42.80±4.55	0.80±0.30		
500 mg/kg BW	115.00±10.86	43.40±7.73	1.18±0.38		
1000 mg/kg BW	93.00±10.12	44.00±4.95	1.19±0.53		
3000 mg/kg BW	100.60±10.36	45.80±12.26	0.78±0.38		
Satellite (control)	115.00±26.87	43.00±7.94	0.70±0.14		
Satellite	121.20±12.83	44.80±7.95	0.59±0.12		
(3000 mg/kg BW)					

The data are expressed as mean±SD, *p<0.05 n=5. SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, BW: Body weight

Table 2: Organ to BW ratio and organ volume ratio (liver and lung)

Groups	Organ to body weight ratio		Organ volume to BW ratio	
	Liver	Lung	Liver	Lung
Control	3.79±0.41	0.93±0.17	3.12±0.60	1.19±0.46
500 mg/kg BW	3.83±0.10	1.32±0.44	3.35±0.37	1.04±0.22
1000 mg/kg BW	4.18±0.42	1.24±0.34	4.00±0.76	1.13±0.38
3000 mg/kg BW	4.29±0.39	1.19±0.20	3.77±0.31	0.81±0.27
Satellite	3.92±0.63	1.33±0.19	3.37±0.36	0.98±0.20
(control)				
Satellite	3.99±0.35	1.31±0.46	2.84±0.56	0.71±0.14
(3000 mg/kg BW)				

The data are expressed as mean±SD, n=5. BW: Body weight

Table 3: Histopathological test of liver and lungs

Groups	Histopathology	Histopathology score	
	Liver	Lung	
Control	0.52±0.11	0.88±0.11	
500 mg/kg BW	0.60 ± 0.14	0.88±0.11	
1000 mg/kg BW	1.64±0.09*	1.44±0.17*	
3000 mg/kg BW	1.64±0.09*	1.40±0.13*	
Satellite (control)	0.64±0.17	0.80 ± 0.11	
Satellite (3000 mg/kg BW)	1.44±0.26#	1.37±0.15#	

The data are expressed as mean±SD, n=5, *p<0.05 compared to the control group, *p<0.05 compared to the satellite group (control). BW: Body weight

Based on the results as shown in Table 2, there was no significant difference in the ratio of lung weight and lungs volume to body weight between the treatment groups compared to the control group. Thus, it could be stated that giving S. androgynous leaves soup did not cause changes in lung weight or volume. This ratio is a sensitive and consistent guide to the body's vital organs including the liver and lungs in describing the body's physiological processes related to metabolic processes. The presence of toxic compounds will damage cells by damaging cell membranes which can cause coagulation of proteins and nuclei so that organ and metabolic function disruption will affect the weight of organs [14]. Observation results on the ratio of liver weight to BW did not show significant differences in the treatment groups compared to the control group. However, the ratio of liver volume to BW in the 3000 mg/kg BW group was significantly different from the satellite group 3000 mg/kg BW. It indicated the possibility of reversibility effects on liver volume, where termination of S. androgynous leaves soup supply for 14 days could reduce toxic substances so that the damage decreased, and liver volume also decreased. Although it could not be concluded, the emergence of toxic effects which had a direct impact on the liver organ as a whole, so it was necessary to do histopathological examination microscopically.

As shown in Table 3, the observation results of hepatic organ histopathology value showed that in the group of 1000 mg/kg BW and the dose of 3000 mg/kg BW had the highest score compared to other treatment groups with an average value of 1.64, which meant liver cell damage ranged from 1-30%. It stated that the dose of *S. androgynus* leaves soup given was not strong enough to cause subchronic toxicity. On the other hand, the highest lung organ histopathology value occurred in the group of 1000mg/kg BW dose (1.44), 3000 mg/kg BW (1.40), and satellite 3000 mg/kg BW (1.36). This means that lung organ damage ranges from 1% to 30%, which also applied in the liver that the dose of *S. androgynus* leaves soup was not strong enough to cause subchronic toxicity.

Liver is an organ which metabolizes compounds that enter the body including toxic compounds so that the liver is sensitive to exposure of toxic compounds. Therefore, changes in the weight ratio and volume of the liver organ are also a sign of toxicity. Normal liver cells as shown in Fig. 2 have a cytoplasm which does not experience swelling and the bright colored nuclei do not experience chromatin clots [13]. If the liver is exposed to toxic substances, special endothelial cells and Kupffer cells in hepatocyte cells which act as macrophages will phagocytose the toxic substance, and if exposure occurs continuously, it will cause reversible (degeneration) as well as irreversible (necrosis) liver damage [15].

In liver tissue preparat as shown in Fig. 3, there was a small damage in the form of cell degeneration found in the portal area. This area has a lot of capillary blood vessels, so it becomes a place for blood to flow from the portal vein to the hepatic vein. Toxic substances are carried by the bloodstream so that if it is exposed continuously, it will be damaged. Liver cells undergo degenerative fatty phase (hydropic) which occurs when tissue damage is heavier and takes longer, characterized by intracytoplasmic isolation. This vacuole can enlarge and force the nucleus cell to the edge of the liver cell [16].

Different conditions were also seen in lung tissue preparate where abnormality was occurred in alveolar. Fig. 4 shows the histology of the lung tissue under the normal condition that the alveoli have a septum covered by a flat layer epithelium. However, in lung tissue preparat as shown in Fig. 5, it was found that the alveoli septa were thickened due to the proliferation of alveolar epithelial cells and also endothelial cells from the blood capillaries. It caused the oxygen absorption process to be disrupted. The use of S. androgynus leaves for a long time can cause bronchiolitis obliterans so that any changes in weight and volume of pulmonary organs can cause a damage due to toxicity of S. androgynus leaves. Inflammation of the bronchioles was characterized by infiltration of neutrophils and lymphocytes around the bronchioles. Inside the lumen of the bronchioles is often found inflammatory cells, erythrocytes, and also cell debris mixed with exudates. In other places, the bronchioles sometimes narrow down and are often followed by fibrosis in chronic cases [17].

CONCLUSION

In this study, the administration of *S. androgynus* leaves soup for 28 days in female Wistar rats did not show any significant toxic effects on the parameters of physical conditions and some clinical biochemical parameters, although several pathological conditions were identified through liver and lung histopathology examination. The results of this study are expected to be the information source about the safety profile of *S. androgynus* leaves soup consumption, where the use of herbs for nursing mothers needs to be pain attention both in terms of its pharmacological and toxicological aspects.

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AUTHORS' CONTRIBUTIONS

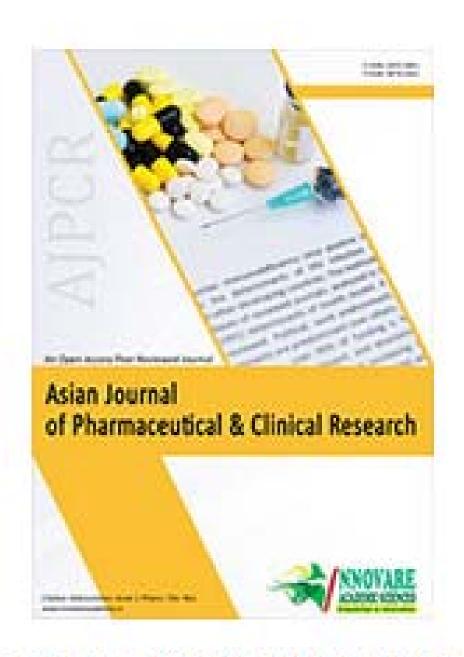
Aguslina Kirtishanti conducted the experiments and also reviewed the manuscript. Ridho Islamie was also conducted the experiments and involved in planning of the experimental research and assisting in the manuscript preparation and data interpretation. The manuscript of the research was done by both of the authors.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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MEDICINE COMPARATIVE STUDY OF WOUND HEALING ACTIVITY OF INDIAN MEDICINAL PLANT AND HERB AGAINST DIABETIC FOOT ULCER (PIPER NIGRUM AND ARACHIS HYPOGAEA)

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