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Acute Toxicity of Mistletoe Tea (*Scurrula atropurpurea* BL. Dans) Ethanol Extract

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ABSTRACT

Mistletoe tea or tea epiphyte is a parasitic plant that lives on tea (*Thea sinensis* L). From generation to generation, tea epiphyte have been used to cure illness such as cough, cancer, diuretics, pain relievers, and can be used to help childbirth. It is necessary to test the security of tea epiphyte through acute toxicity test in order to obtain an overview of therapeutic index. Aim of this studty : to determine acute toxicity of ethanol extract of the mistletoe tea expressed in LD50 values. Simplicia extracted using Soxhlet equipment with 96% ethanol. Fractionation was conducted using liquid-liquid extraction using a solvent of water, ethyl acetate and n-hexane. The ethanol extrtact and fractions were subject to phytochemical screening. Acute toxicity of the tea epiphyte expressed in LD50 values which was calculated by using log probit method. Animal that was used on this experiment was Swiss webster strained mice. The mice were divided into two major groups, including the control group (PGA 2%) and a test group that would be given extract suspensions with dose variations orally. The results of toxicity tests showed that LD50 value of the ethanol extract of tea parasite in the male is 6,88 g /kg while the female mice of 5,74 g /kg in mice. The results of pharmacological screening showed that administration of the test preparation may influence the effect on body weight of mice male and female, motor effects on male mice, the effects of catalepsy in male mice, the effects of flexion in male mice, and the Hafner effects on male mice. The mistletoe tea, scurrula atropurpure ethanol extract based on the criteria of toxicity in the category of non-toxic ie> 15g / kg rats.

Keywords: Mistletoe tea, Scurrula atropurpurea, acute toxicity, log probit, thea sinensis.

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INTRODUCTION

Indonesia is a country with abundant natural resources, in which there are thousands of types of plants that can be used as a medicinal plant. Since time immemorial, the people of Indonesia have known medicinal plants and apply it in the treatment of disease. This makes many researchers are interested to test and prove the efficacy of medicinal plants scientifically. One example of this is mistletoe tea or tea epiphyte (*Scurrula atropurpurea* BL. Dans). Tea epiphyte is a plant parasite on the tea tree (*Thea sinensis* L), and the extract has been reported to have 16 of bioactive material [1]. Empirically, parasite tea is used as a cough medicine, cancer, diuretics, pain relievers, and care after childbirth [2]. According to Hotimah [3]. this type of mistletoe tea can also be used for the treatment of hypercholesterolemia. Research of Muwarni [4] states that the parasite tea can destroy tumor cells directly, and make it more sensitive to molecular tumor-necrosis-factor-alpha (TNFα). Some other research has published that mistletoe tea can improve the immune system [1] and can inhibit the growth of tumor cells ([5].

Various series of studies on tea parasite has been reported empirically, in vitro and in vivo. Empirical research conducted on crops tea parasite Viscum album kind of potential to lower blood pressure [6-10].This study was followed by other researcher [11] in vitro using parasite tea types Scurrula oortiana and to reduce the contractility of the arteries of mice. Invitro study was continued in vivo in mice in DOCA-salt hypertensive exposure to the parasite tea sample types Scurrula atropurpurea. This results showed a decrease of pressure blood in test animals [12-13]. In contrast to these researchers, here we reported the acute toxicity of ethanol extract of mistletoe tea which is part of all our research that aims to make pharmaceutical preparations for the adjuvant treatment of breast cancer from plants S. atropurpurea.

MATERIALS AND METHODS

Plant materials:

Fresh samples (mistletoe Scurrula atropurpurea) was collected in sufficient quantities (~ 10 kg) at a time [14] in order to ensure the sample was from the same source throughout the experiment. Samples were taken from the area of Subang, West Java. First, the plant was washed with tap water, followed by rinsing with distilled water and then each piece cut into small pieces and powder. They were dried by the sun (~ 30 ° C) in open areas with active ventilation until they reached a constant weight (about a month).

Experimental animals:

Male and female mice (Mus musculus) weighing between 20 to 30 g and 2-3 months of age, obtained from the School of Pharmacy, Institute of Technology Bandung, Indonesia. Before use, the mice adjusting for one week and their body weight was measured every day. Mice were said to be healthy if the weight was increased or reduced by no more than10% of the previous weight with normal activities during acclimation period.This was subjected to ethical permission from the Faculty of Medicine, University of Padjadjaran, Health Research Ethics Committee.

Extraction method

Soxhlet apparatus used for the extraction process were installed between the extractor thimble round bottom flask on the bottom and a condenser at the top of the ball. In the thimble holder, 200 g of dried S. atropurpurea powder wrapped in a package. 1.8 L extraction using 76% ethanol was applied to get a clear droplets. This procedure was guided by the Indonesian Herbal Pharmacopoeia [15] and modified Gatbonton [16] and Marnoto [17] methods. The extract obtained was concentrated using a rotary evaporator and freeze dryers. The extract obtained was divided into four main parts, namely for phytochemical screening, determination of moisture content, ash content determination and thin layer chromatography.

Acute toxicity method

Acute toxicity testing methods were similar to our previous method which was based on modified Ouedraogo et al . method and The OECD Guideline Procedure [18-20]. Acute toxicity tests carried out within 14 days. Animals were randomly divided into five groups of five mice each. The group given the test

material with different doses, one group was given a liquid carrier as a negative groups. Each test dose given orally. After administration of the extract, the observed number of mortalities of each test group every 1/2 hour, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 24 hours, up to 14 days. Data were analyzed using probit analysis method, and then determined its LD50 value. Body weight of mice were weighed every day. Observation of the emergence of symptoms of toxic mainly symptoms of abnormal behavior, symptoms of tremors, convulsions, motor incoordination, pineal reflex, catalepsy, piloerection, lacrimation and Straub.

Data Analysis

Data were statistically analyzed using the Analysis of Variance (Anova) with methods One-way ANOVA followed by Tukey's test if there were differences in treatment effect in the test group after they were given suspensions extract tea parasite (Scurrula atropurpurea BL. Dans) in PGA 2%.

RESULTS AND DISCUSSION

Extraction

Extraction S. atropurpurea (300 g) with soxhletation with 96% ethanol obtained from 35.4 g condensed extract with the results of 11.80%. The water content in tea mistletoe obtained at 4.90%. The water content was set to maintain the quality of the extract. Based on Indonesian Herbal Pharmacopea [15], the water content in the extract should not be more than 10% aiming to avoid the rapid growth of fungi and microorganisms in the extract.

Fractionation

Tea parasite extract ethanol fractionation with liquid-liquid extraction method obtained three fractions of the water fraction, fraction of ethyl acetate and n-hexane fraction of the weight of each fraction condensed 4.50 g, 2.35 g and 2.75 g. The percentage of water fraction, fraction of ethyl acetate and n-hexane fraction were 2.26%, 1.16% and 1.35%, respectively.

Phytochemical screening

Phytochemical screening results are shown in Table 1.

Scurrula species has not been extensively studied in terms of phytochemicals [21]. Avaibility of flavonols in the species Scurrula reported by three articles [21-23]. Lohézic-Le Dévéhat et al. [24] successfully isolated from Scurrula ferruginea three flavonols: quercetin, quercitrin, and 4 "-O-acetylquercitrin and acetate derivatives latter is rare in higher plants. Priyanto et al. [25] stated the ethanol extract of Scurrula atropurpurea consisted of flavonoids quercetin. Allegedly quercetin expressed using the color reagent magnesium powder, HCl, and added amyl alcohol and shape of red, yellow, or orange on amyl alcohol showed flavonoids. this reaction was actually a pointer reaction to the flavonoids in phytochemical screening.

Our research revealed showed that the ethanol extract, fractions of water, and ethyl acetate contain flavonoids and polyphenols that have the potential as antioxidants. This assumption is in-line with others who claimed the majority of antioxidant compounds present in these plants are secondary metabolites-phenolic (tannins, flavonoids, and phenolic acids) that shows them through the protective properties of free radicals [26-28]. In addition to phenolics, the plant can also contain essential oils, carotene, and vitamin [29]

Table 1. Phytochemical screening of Scurrula atropurpurea

No	Secondary metabolite	Simplicia	Ethanol extract	Water fraction	Ethyl acetate fraction	n-hexane fraction
1	Alkaloids	-	-	-	-	-
2	Polyphenols	+	+	+	+	-

3	Tannins	+	+	-	+	-
4	Flavonoids	+	+	+	+	-
5	Monoterpenoid and sesquiterpenoids	+	+	+	+	-
6	Steroids	+	+	+	+	+
7	Triterpenoids	+	+	+	+	+
8	Quinones	+	+	-	+	-
9	Saponins	-	-	-	-	-

+ : detected

-: not detected

Thin layer chromatography (TLC)

It was found that results TLC using developer: toluene: ethyl acetate: acetic acid (5: 4: 1) showed that the ethanol extract, fraction of ethyl acetate, and n-hexane fraction had three spots while the water fraction had one spot. On the results of TLC with DPPH reagent, the color changes to yellow with purple background color indicated the presence of compounds that have antioxidant activity. In extracts of ethanol, water fraction, fraction of ethyl acetate, and n-hexane fraction, visible yellow color of the compound spotted with purple background meaning that extracts and fractions contain compounds that have antioxidant activity. Profile of each spots and Rf value of chromatography results is shown in Table 2. Unlike this study, Krisnawati [30] used a two-dimensional paper chromatography to isolate the flavonoid extract from ethanol to butanol: acetic acid: water (3: 1: 1). They partially determined the structure of flavonoids by UV-visible spectroscopy with different diagnostic reagents.

Table 2 TLC Profile of S.atropurpurea ethanol extracts

Sample	Spotting no.	Rf value	Visible light	UV 254 nm	UV 366 nm	DPPH reagent
Ethanol extract	1	0.08	Brown	Purple	-	Yellow
	2	0.5	-	Purple	-	Yellow
	3	0.93	-	Purple	Purple	Yellow
Water fraction	1	0.95	-	Purple	Purple	-
Ethyl acetate fraction	1	0.06	Brown	Purple	Green	Yellow
	2	0.75	-	Yellow	Red	Yellow
	3	0.93	-	Yellow	Red	Green
n-hexane fraction	1	0.94	Brown	Purple	-	Yellow
	2	0.75	Brown	Purple	Purple	Yellow
	3	0.95	-	Purple	Green	-

DPPH: 2,2-diphenyl-1-picrylhydrazyl, UV: Ultrviolet

Acute toxicity

Acute toxicity was evaluated through observation of death in the mice for 14 days, the observation of the weight of mice for 14 days, and observations of the behavior of the mice in the first 24 hours after being given mistletoe S. atropurpure ethanol extract.. Death fata for male and female mice are reported in Tables 1 and 2, respectiv**ely.**

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Table 1. Male mice mortality (%).

	Total Cumulative Mortality (%) in days													
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Control (PGA 2%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dose I (0.5 g/kg BW)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dose II (1.0 g/kg BW)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dose III (2.0 g/kg BW)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dose IV (4.0 g/kg BW)	0	20	20	20	20	20	20	20	20	20	20	20	20	20
Dose V (8.0 g/kg BW)	0	40	60	60	60	60	60	60	60	60	60	60	60	60

Table 2. Female mice mortality (%)

	Total Cumulative Mortality (%) in days													
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Control (PGA 2%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dose I (0.5 g/kg BW)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dose II (1.0 g/kg BW)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dose III (2.0 g/kg BW)	0	0	0	0	0	0	20	20	20	20	20	20	20	20
Dose IV (4.0 g/kg BW)	0	0	0	0	20	20	20	20	40	40	40	40	40	40
Dose V (8.0 g/kg BW)	0	20	40	40	40	60	60	60	60	60	60	60	60	60

To calculate the value of LD50 data was drawn to a straight line between log dose vs probit percentage value. Probit percentage determined using probit table [31] by changing the value of % of animal deaths to the percentage in the table probit. Having obtained a linear line equation, then the LD50 was calculated by changing the value of Y to 5, the results obtained made his anti-Log [32,33] as shown in Fig 1.

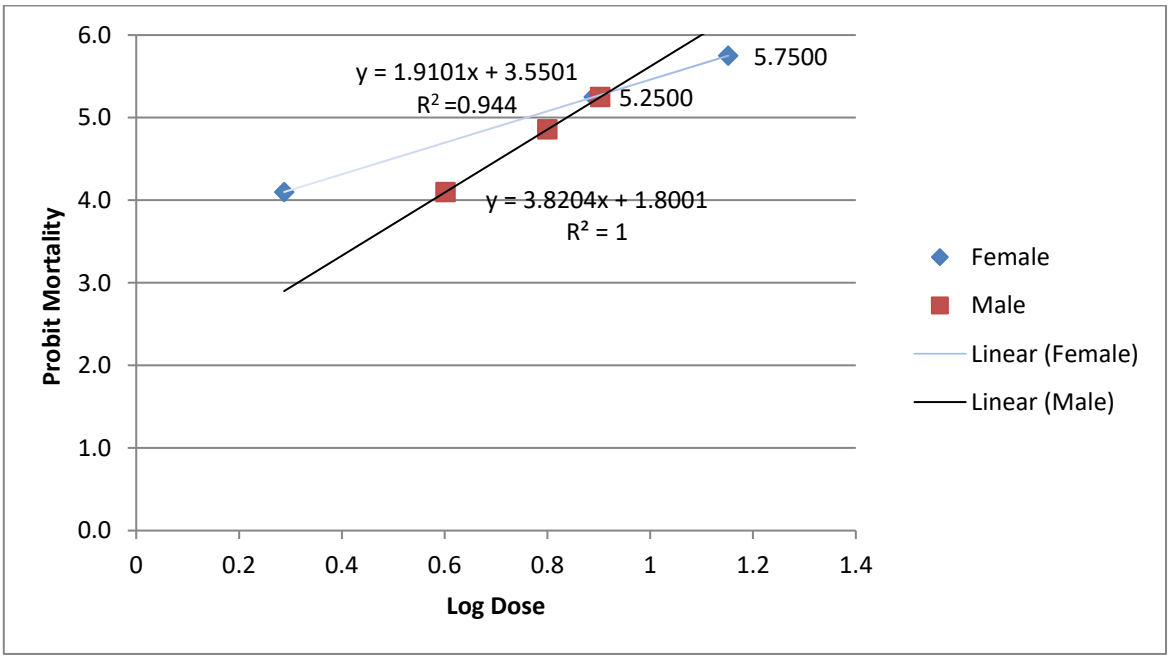

Figure 1. Probit analysis

Curve of blue line shows the cumulative mortality (%) of female mice over the 14 day period, compared with that of the male mice. Based on the Figure 1, it was found male and female mice had LD of 6.88 and 5.74 g/kg BW, respectively. According to the criteria of Hodge and Sterner (Table 3) [34], the results of toxicology LD50 value has meaning and it could be concluded that the ethanol extract S. atropurpurea in the range of doses was practically not toxic. These findings are very similar to our previous report on the acute toxicity of mistletoe mango Dendropthtoe pentandra [18].

Table 3. Substance toxicity assessment [34}

Toxicity levels	General terms	LD50in rats (orally)
1	Unusually toxic	≤1 mg/kg
2	Very toxic	1–50 mg/kg
3	Quite toxic	50–500 mg/kg
4	Slightly toxic	0,5–5 g/kg
5	Practically non-toxic	5–15 g/kg
6	Relatively safe	>15 gkg

Observations on Body Weight

Daily body weight was observed for 14 days in both the male and female mice. This was conducted to determine if the extract had any effect on weight for the two week period following administration. These data were analyzed using analysis of variance (ANOVA). The results of the statistical testing demonstrated that there was no significant difference in body weight of the male and female mice as a result of the administration of different doses of the extract at a significance level of 0.05.

Observations Pharmacological screening

Pharmacological Screening is done to get a clear picture of the effects of drugs on the body so that it can be seen the possibility of toxic effects that arise and provide direction for further research. Screening pharmacology observed in ½, 1, 2, 4, and 24 hours after administration of the test perfomed. Observations made include symptoms of abnormal behavior, symptoms of tremors, hanging, convulsions, motor incoordination, pineal reflex, catalepsy, piloerection, lacrimation and Straub.

Motor effect

Motor effect or motor incoordination was spontaneous activity which was muted response when mice were put into the bottle show curiosity. By using Kruskal Wallis method obtained p-value 0.00 <0.05, which meant that H0 was rejected, so that there was a significant influence on the use of samples with dose variation in male mice so that the test followed by Tukey method. From the Tukey test, it was found that there were significant variations in the dose of the motor effects of male rats. In female mice, however, showed motor effects of p-value 0.881> 0.05 (a significance level of alpha) which meant that H0 was accepted, so there wass no significant effect on the differences in the administration of the test preparation dose variation on female mice. This result as well as LD50 results is consistent with the theory put forward by Lazarovici and Haya [35] which states that between males and females there are differences in sensitivity to a toxicant. The differences are influenced directly by the endocrine glands,

Hanging affect

Hanging effect was a state of activity of mice hanging wire after administration of the test material. Observation of the hanging effect performed to test the ability of locomotor coordination seen from the tendency of mice to pursue strands can run on test equipment. Giving the test preparation may decrease hanging ability in mice, except the control preparation. Test results by Kruskal Wallis method, hanging effects on male and female mice showed each p-value 0.27> 0.05 and 0784> 0.05. It could be concluded that there was no significant hanging effect on the administration of the test preparation with dose variation against both types of mice. Procedures regarding the hanging effect also done other researchers [36-37].

Other effects

Other effects include retablismen, flection, Hafner, pineal, respiratory, giving cataleption and sedative effect, the effect of the autonomic nervous system: piloerection was also conducted using statistical methods and Kruskal Wallisretablismen, flection, hafner, pineal, respiratory, giving cataleption and sedative effect. Effect of autonomic nervous system: piloerection. Other researchers who conduct research on acute toxicity usually also do other research on the effect of these outcomes [38-40].

Observation of the effect restablismen performed to test the ability of locomotor coordination seen from the ability of mice to return to the normal position of a hanging position. Kruskal Wallis test results restablismen effects on male and female mice respectively showed the p-value 0.465> 0.05 and p-value 0.807> 0.05, which meant that H0 was accepted, so there was no significant effect on the administration of the test preparation with variations dose to male and female mice.

Catalepsy effect that the state of maintenance of posture but for an indeterminate length of time, which can be seen from the posture mice to follow the movement of props when lifted her body through both front legs. Catalepsy test was observed to determine the sensitivity and reflex mice to a treatment. Sedative effect is characterized by a decrease in activity, vigor, and causing drowsiness. Testing is done with sedation turned mice if it starts to look sleep. If there is movement or reflex of mice, then test sedative declared negative. Kruskal Wallis test results catalepsy and sedative effects in male mice showed 0.02 p-value <0.05 (significance level of alpha) which meant that H0 was rejected, so that there was a difference significant effect on the administration of the test preparation with dose variation on male mice. Then conducted further tests using Tukey. Results of the Tukey test showed that all treatments did not provide a significant difference, it was contrary to the Kruskal Wallis results before, and therefore carried out further tests with the method of Duncan. Duncan on the test results, a dose of I, II, III, IV, and dosage control gives the same effect on the treatment in the first subset. Dose IV and V give the same effect to the treatment in the second subset, with no significant difference between the two subsets. To female mice, it was found that there was no significant effect on the administration of the test preparation with a variety of doses.

Effect tremor was a state of vibration or chills (shaking body parts that were not controlled) was characterized by mice body trembling. Convulsive effects characterized by symptoms of seizures due to the severe contraction that was not desired. Kruskal Wallis test results for the effects of tremors and convulsions in both male and female mice showed significant effect on the administration of the test preparation with dose variation on both mice.

Straub effect was a state of pain in certain parts of the body marked with mice became strained. Kruskal Wallis test resulted Straub effects on both male and female mice showed there was no significant effect on the administration of the test preparation with dose variation on male mice.

Tests on the effects of flexion was the response that arised when mice were clamped at the tail. The test was declared negative if the mouse did not seem to feel pain when done stapling option at the tail. Kruskal Wallis test results flexion effects on male mice showed 0.03 p-value <0.05 (significance level of alpha) which meant that H0 is rejected, so that there is a difference significant effect on the administration of the test preparation with dose variation on male mice. Then conducted further tests using Tukey. As results of the Tukey test showed contrary to the Kruskal Wallis did before, and therefore carried out further tests with the method of Duncan. Duncan on the test results, a dose of I, II, III, IV, and dosage control gave the same effect on the treatment in the first subset. Dose IV and V give the same effect to the treatment in the second subset,

with a significant difference between the two subsets. To female mice there was no significant effect on the administration of the test preparation with a variety of doses.

Tests conducted on the effects Hafner unuk know the response that arised when mice were clamped on the legs. The test was declared negative if the mouse did not seem to feel pain when done stapling option at the foot. As in the case of flexion effect, Kruskal Wallis test results Hafner effects on male mice required Turkey and Duncan method to show confirmed results. To female mice there was no significant effect on the administration of the test preparation with a variety of doses.

Tests on the effect of the pineal do transform and evaluate the response that arised when mice were clamped on the ears. The test was declared negative if the mouse did not seem to feel pain when done stapling option at the ear. Kruskal Wallis test resulted pineal effects on male and female mice respectively show the p-value 0.464> 0.05 and p-value 0.380> 0.05 meaning there was no significant effect on the administration of the test preparation with a variety of doses.

Respiratory effects testing conducted to determine the effect of the test preparation to the mice breathing. Observation of the effects of breathing characterized by the body of mice were panting. Kruskal Wallis test results effects in male and female mice showed p-value 0.489> 0.05 on female mice showed p-value 0.750> 0.05 which meant that H0 was accepted, so there was no significant effect on the administration of the test preparation with a variety of doses to female mice.

Observation on Piloerection effect where there was a contraction in the erectile tissue of the hair follicle so the hair becomes rough, effect salivation, lacrimation, and urination, and diarrhea effect resulted no significant effect on the administration of the test preparation with a variety of doses.

CONCLUSIONS

From the results of acute toxicity testing of 96% ethanol extract of tea parasite in male and female mice using probit analysis LD50 values obtained in male mice of tea parasite extract was 6,88 gr/kg in mice. While the value of LD50 in female mice were 5,74 gr/kg in mice. Based on the classification of toxicity, the toxicity of tea parasite extract categorized as practically non toxic doses are in the area of 5-15 g/kg body weight of mice. Pharmacological screening test showed that administration of the test preparation may influence the effect on body weight male and female mice, motor effects on male mice, the effects of catalepsy in male mice, the effect of flexion on male mice, and the Hafner effects on male mice.

To complete the research that has been done, it is advisable to test the toxicity and chronic and sub-chronic toxicity test specific to supplement the data concerning the safety of 96% ethanol extract of mistletoe tea to be a herbal medicine.

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