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Characterization and Sub Acute toxicity Ethanol Extracts from Leaves of coffee Parasites (*Scurrula ferruginea* Jack Dance) to the activity of SGPT and Serum Creatinine Levels male white mice.

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ABSTRACT

This study has been conducted to characterize and test sub-acute toxicity of the ethanol extract of coffee parasite leaves (*Scurrula ferruginea* Jack Danser) to the activities of the alanine aminotransferase (SGPT) and serum creatinine level of male white mice. The animals were divided into 4 groups consisting of one as a control group and three as test groups. The extract was given orally for 7, 14, 21, days with dose of 100, 200, and 400 mg/kg/BW. Blood was collected from the veins of the neck, then the blood was centrifuged to collect serum for the test. To determine the function of liver, it could be seen through the SGPT activity, and to determine the function of kidneys, it could be seen through the serum creatinine level. Data were analyzed by two way ANOVA followed by *Duncan's Multiple Range Test*. The results showed that the ethanol extract of the coffee parasite leaves at dosage of 100, 200 and 400 mg/kg/BW showed significant effect on the increased activity of the alanine aminotransferase (SGPT) and serum creatinine levels of male white mice ($p < 0.05$).

Key words: sub-acute toxicity, *Scurrula ferruginea,* ethanol extract, SGPT, creatinine serum.

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INTRODUCTION

Traditional medicine is one of the cultural heritages of Indonesian nation used to look after, increased of the health, prevent and cure diseases 1]. One of the traditional medicines used by the society is the coffee parasite (*Scurrula ferruginea).* This herb has other names that is *Loranthus ferrugineus* Roxb, *Taxillus ferrugineus* Roxb[2][3]. Traditionally this herb can be used as antiviral, anticancer [4] antioxidant, antimicrobe, and to cure hypertension [2][5]. On use of traditional medicines, in fact, can also cause unexpected effects. This is caused by the dose and the length of time the use of traditional medicines is not appropriate [6]. Test the safe use of medicines is necessary to secure the safety and benefits, so that it can be registered as fitofarmaka and safe to use [7].

Test on safety can be conducted through several studies, among others study of acute toxicity, sub acute toxicity, sub chronic and chronic toxicity [8]. One of the observations is focused on toxicity test organ function, such as the liver and kidneys. All of the chemicals that enter the body will pass through various types of processes in the body, including metabolism, and excretion. Metabolism process occurs in the liver. If liver cells are contaminated by toxic substances in the dose and duration of time, liver cells will be damaged, so that the enzymes in the cell is reduced and the rising levels in the blood. The change of enzyme level of the liver in the blood can be used as an indicator to identify the liver damage [9]. One of the enzymes produced by liver is that *Glutamic Pyruvic Transaminase* (GPT). Enzymes Glutamic Pyruvic transaminase (SGPT) is normally located in the cytoplasm, mostly in the liver cells and also in blood cells. If the cells are damaged, the lysis (damaging cells by a certain substance) will happen. GPT enzymes will be out of the cell and enter into the serum. The existence of enzymes in the serum is used as an indicator of the cell damage, especially the liver cell damage [10].

MATERIALS AND METHODS

The materials used in this study were *Scurrula ferruginea* herbal leaves and organic solvents, such as 70% ethanol, quail egg yolk, Na CMC 0,5%, the analysis of reagent SGPT and creatinine analysis reagents.

Herbal identification and fitochemical examination were conducted in ANDA Laboratory, Andalas University. The coffee parasite leaves were dried out in the breeze and then sliced, followed by maceration to make the ethanol extract. 1 kg sample of dried parasite leaves was soaked and stirred in ethanol 70% for 6 hours and left 18 hours before filtration and distilling. After that a rotary evaporator was used to thicken the sample.

The method used had the experimental characteristics. The research design was conducted as following: taking sample of coffee parasite leaves (*Scurrula ferruginea)* , sample identification, production of ethanol extract of coffee parasite leaves, characterization of ethanol extract of coffee parasite leaves, readiness of experimental animals, designing dosage and grouping the animals, making readiness of tests, treatment on experimental animals, test on the ethanol extract of coffee parasite leaves on liver function with the determination SGPT activity, test on the ethanol extract of coffee parasite

leaves on kidney function with the determination of serum creatinine level and data operation and analysis.

We used 12 healthy male white mice with 20 – 30 grams of weight. The mice were divided into four groups, one as a control group and the rest as groups of treatment. All mice were acclimatized for 7 days prior to experiment. The experiment was conducted in 21 days [11][12].

For the experiment the male white mice were chosen regarding to economical, easy to find, tackle, and almost the same physiology of human. Since the cholesterol level was influenced, by genetic characteristic age, gender, body weight, and environments, we chose the mice with similar channel, sex, age, and almost the same body weight to minimize the diversion of the result of the study. Male mice were used to avoid the influence of hormonal factor [11][12].

The Suspension was made as following: 500 grams mg of Na CMC was sown on 20 L hot water then left it for 15 minutes. The Na CMC then was scrapped and distilled in 100 mL distilled water.

The ethanol extract of *Scurrula ferruginea* Jack Dancer suspended by using Na CMC 0.5% B / W in different concentrations. Doses used for examination were (100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW). The range of doses was determined by Thomson formula 0.1% BW of male white mice, with three kind of concentration: 1%, 2% and 4%.

The experimental animals were randomly divided into 4 groups. Each group consisted of 9 male white mice. Group 1 was as a control group given only the essential substance, and group 2, 3, and 4 as the test groups that were given the test readiness with the series of dosage of 100 mg/kgBW, 200 mg/kgBW and 400 mg/kgBW. This readiness was orally given once a day for 7, 14 and 21 days. On day 8^{th}, 15^{th} and 22^{nd}. The mice were victimized by cutting the part of the neck, the blood was collected in the micro tubes. The blood was left for 10 minutes then the serum was collected by rotating the blood with the speed of 3000 rpm [13]. The serum was separated by using micropipette. The serum could be used to determine SGPT activity and creatinine serum.

Tests on the Effects of the Ethanol Extract of Coffee Parasite Leaves on the Determination SGPT Activity

The serum total of 100 µL (0.1 mL) was pipetted and put into the test tube and added 1 mL of reagen 1, then put in a vortex and left for 3 minutes. After that reagen 2 as much as 0.25 mL was added and put in a vortex. After 1 minute the absorption was measured with spectrophotometer UV visible in the wave length of 365 nm for 3 minutes, then the different average absorption was counted every minutes.

SGPT activity can be counted with formula:

SGPT activity (U/L) = AA/minute x F

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Explanation: AA minute : average absorption change .

$$AA/\text{ minute} = \frac{(\text{Abs sample 2} - \text{Abs sample 1}) + (\text{Abs sample 3} - \text{Abs sample 2})}{2}$$

Abs sample 1 = sample absorbency in the first minute
Abs sample 2 = sample absorbency in the second minute
Abs sample 1 = sample absorbency in the third minute
F = factor 3235 (for measuring in the wave length of 365 nm)

Test on the Effect of Coffee Parasite Leaves on the Function of Kidneys with the Determination of Serum Creatinine Level

As much as 50 µL serum was pipette, put in a test tube. 1 mL of regen 1 was added, put in a vortex and left for 3 minutes. Then 0.25 mL of reagen was added and put in the vortex. The sample absorbency was measured in the first and third minutes using spectrophotometer UV visible in the wave length of 492 nm. Serum creatinine Level could be determined using the following formula:

$$Scr = \frac{As2 - As1}{Ast2 - Ast1} \times 2 \text{ mg/dL}$$

Explanation:

Scr	= Creatinine level in the serum (mg.dL)
As1	= Sample absorbency at the first minute
As2	= Sample absorbency at the third minute
Ast1	= Standard creatinine solution absorbency at the first minute
Ast2	= Standard creatinine solution absorbency at the third minute
2 mg/dL	= The concentration of standard creatinine solution

The data of this study were analysed using two way ANOVA followed by Duncan (Duncan's Multiple Range Test) to see the significant difference among each treatment group.

RESULT AND DUSCUSSION

Characterization of the Extract of Coffee Parasite Leaves

From 10 kg of fresh sample of coffee Parasite leaves it only had 2545 g dry sample. The extraction process with maceration method using the ethanol 70% for 24 hours with twice repetitions and thick extract of 173.1538 g was obtained, The result of characterization of the thick extract of coffee parasite leaves including organoleptic, *rendemen* (6.8036 %), decrease of dryness (20.420 %) and dust level (6.14 %).

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KLT Profile

In the KLT profile using eluent ethylen acetic: methanol 9:1 there was a stain under the UV 254 nm with its Rf 0.66 (Table 1,Picture 1).

Picture 1. KLT of ethanol extract of coffee parasite leaves

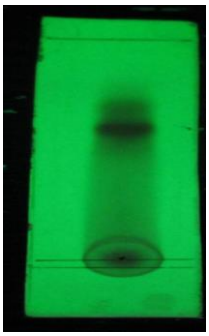

Table 1. Rf value of coffee parasite coffee parasite leaves

Eluen	Plat length	Stain length	Rf
Ethylen acetic: methanol 9:1	4.5 cm	3 cm	0.66

Test on the Ethanol Extract of Coffee Parasite Leaves on the SGPT Activity of the Male White Mice

The SGPT activity was affected significantly by the dose ($p<0.05$) and was not affected significantly by the length of time of giving ($p>0.05$). There was not any significant effect of the interaction between doses and the length of time of giving the SGPT level ($p>0.05$). The mean of SGPT activity on the control group and that given readiness test with the dosage of 100mg/kg/BW, 200mg/kg/BW and 400mg/kg/BW were 28.397±1.893, 48.525±2.788, 49.782±3.502 and 70.982±4.021 U/L. And for the average of SGPT activity on the 8^{th}, 15^{th} and 22^{nd} were 51.086±17,611, 50.547±18.115 and 46.633±18.887 µL (Table 2).

Tabel 2. The Effect of Dosage and the Length of Time of Giving the Ethanol Extract of Coffee Parasite Leaves on Male White Mouse SGPT Activity

	SGPT Activity on			Activity
				SGPT Average (uL)
Dosage	8	15	22	
Control	31.271±4.211	28.575±3.536	25.345±2.858	28.397±1.983
100 mg/kg/BW	46.907±4.941	48.526±7.050	50.142±4.070	48.525±2.788
200 mg/kg/BW	51.965±3.422	52.299±7.253	44.75±7.943	49.782±3.502
400 mg/kg/BW	81.683±5.661	72.787±5.348	66.293±8.554	7.982±4.021
Average Activity! SGPT (uL)	51.086±17.611	50.547±18.115	46.633±16.887	

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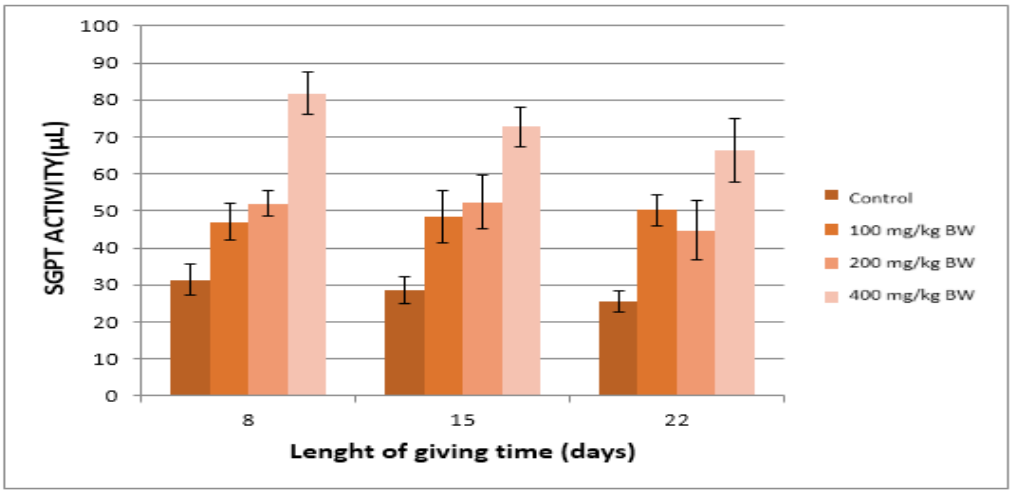
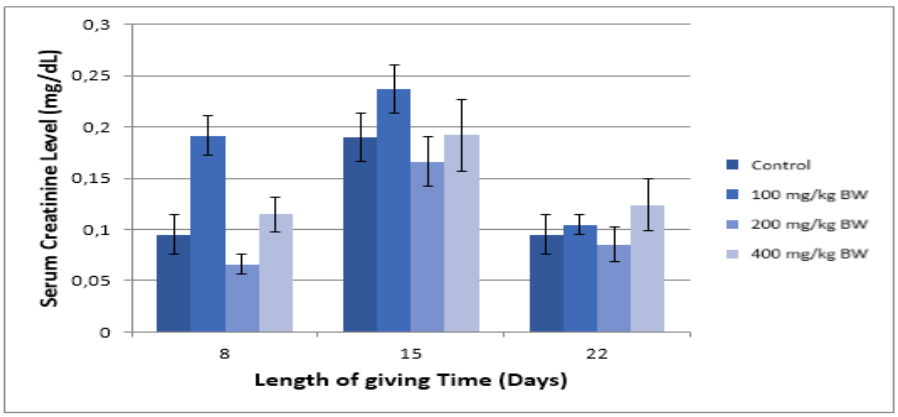


Picture 2. The Effect of Dosage and Length Time of Giving Ethanol Extract of Coffee Parasite Leaves on SGPT Activity

Table 3. The Effect of Dosage and Length of Time of Giving Ethanol Extract of Coffee Parasite Leaves on Serum Creatinine Level of Male White Mice

Dosage	Average Creatinine Level 8	15	22	Average Creatinine Level (mg/dL)
Control	0.0946±0.0193	0.1900±0.0240	0.946±0.0193	0.1264±0.190
100 mg/kg/BW	0.1913±0.0193	0.2373±0.0 233	0.1046±0.0093	1.777±0.0214
200 mg/kg/BW	0.0660±0.0100	0.1660±0.0240	0.0853±0.0167	0.1057±0.0177
400 mg/kg/BW	0/1146±0.0167	0.1920±0.0349	0.1240±0.0253	0.1435±0.0180
Average Creatinine Level (mg/dL)	0,01166±0.0157	0.1963±0.0138	0.1435±0.0090	

Test Results Sub Acute Toxicity Study of Ethanol Extracts Coffee parasite leaves to the Kidney Function of Male White Mice



Picture 3. The Effect of Dosage and Length of Time in Giving the Ethanol Extract of Coffee Parasite Leaves on Serum Creatinine Level

The Serum Creatinine Test on the Ethanol Extract of Coffee Parasite Leaves on the Serum Creatinine Level of Male White Mice

The serum creatinine level was significantly affected by the dosage of ($p<0.05$) and significantly affected by the length of time of giving ($p<0.05$). Meanwhile there was not any effect of interaction between dosages and the length of time of giving on serum creatinine level ($p>0.05$). The average level of the serum creatinine of control group and those given test readiness with dosage of 100mg/kg/BW, 200mg/kg/BW and 400mg/kg/BW successively were 0.1264±0.190, 1.777±0.0214, 0.1057±0.0177, 0.1435±0.0180 mg/dL. The average level of serum creatinine on the 8^{th}, 15^{th} and 22^{nd} successively were 0.01166±0.0157, 0.1963±0.0138, 0.1435±0.0090 mg/dL (Table. 3).

The data as the results of this study were various enough on each of the animals. This could be seen from the graph of average of SGPT activity and average of creatinine in which the graph was seen fluctuated. This mistake could occur during the reading of the results of the study such as the improper way in taking the blood, the improper use of the tube for blood sample so that it could cause lysis in the blood cells, carelessness in taking sample and mixing reagens. Another factor that can influence the results such as providing food for mice, conditions to keep mice, different physiological conditions of mice, mice internal factors such as stress [14].

From the study conducted it can be assumed that the ethanol extract of coffee parasite leaves can affect the SGPT activity and average serum creatinine. When compared with previous studies conducted on the acute toxicity of water extract of leaves of mango parasites LD_{50} data obtained is 16.0962 g/kg. This may cause by the different place of growing of the parasite in spite of that they are still in the same family, different plants used as places of growing and the response of the animals used. From the results of this study, it can be concluded that the coffee parasite leaves can affect to SGPT activity and serum creatinine level. To see the function of kidney, it can be seen from the creatinine level. Creatinine is a muscle separating product released from the muscles by almost constant rate and excreting in the urine in the same rate [15]. Creatinine is excreted by the kidneys through the combination filtration and excretion. In the normal condition creatinine secretion is so little that it can be ignored, but in the condition of a kidney damage, the number of creatinine secretion in tubulus increases [16][17].

Creatinine clearance is counted by measuring urine creatinine level and serum creatinine. But in this study serum creatinine is only counted, because of the difficulty in collecting urine in 24 hours and the lack of instruments for collecting the urine in the laboratory. Besides creatinine concentration in plasm is relatively the same from day to day. So that serum creatinine is an indicator for the kidney function [17].

The statistic calculation on the kidney function with the parameter that will be observed serum creatinine level using two way ANOVA shows that serum creatinine level is affected significantly by dosage factor and the length of time of giving ($p<0.05$). Then further test Duncan's Multiple Range (DMRT) is continued based on the dosage factor, it can be seen that control and dosage 200 mg/kgBW are not distinctly different because they are in the same subset as the average serum creatinine level successively 0.126 and 0,105

mg/dL, It is distinctly different from 100 mg/kg BW and 400 mg/kgBW are distinctly different that are in the second and third subset with the average serum creatinine level successively 0.177 and 0.143 mg dL.

After further test of Duncan's Multiple Range (DMRT) is conducted based on time factor, the average difference of serum creatinine level on the day of 8^{th}, 22^{nd} and 15^{th}. The average serum creatinine level on the day of $8^{th}$and 22^{nd} is on the first subset with the successive value of 0.116 and 0.102 mg/dL. As for the average serum creatinine level on the day of 15^{th} is on the second subset with the value of 0.196 mg/dL.

The average increase of serum creatinine level occurs on the 15^{th} day. This may be caused by several factor such as the use of *sonde* that makes the throat of the mice feel sore when giving the extract, the speeding on of protein metabolism and serum creatinine will increase in the condition of sickness (fever and infection)[18]. The normal value of serum creatinine of mice is 0.2 – 0.9 mg/dL[19]. Seeing the result of the study, the value of the serum creatinine mice is still in the normal condition.

CONCLUSION

From the study having been conducted it can be concluded that:

- Giving the ethanol extract of coffee parasite leaves in the dosages used, give the significant effect on SGPT activity and serum creatinine level ($p<0.05$).
- The length of time of giving the ethanol extract of coffee parasite leaves gives effect on the serum creatinine level ($p<0.05$).

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