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Oral Subchronic Toxicity Test of Yellow Root Ethanol Extract (*Arcangelisia flava* Merr.) in Mice (*Mus musculus*)

(*Uji toksisitas subkronik oral ekstrak etanol akar kuning (Arcangelisia flava* Merr.*) pada Mencit (Mus musculus)*)

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ABSTRACT

Background: Yellow root (*Arcangelisia flava Merr.*) is a native Indonesian plant that contains alkaloids, phenolics, flavonoids, saponins, tannins and berberine. This plant is proven to have antimicrobial, antioxidant, antihyperlipidemic, and anticancer activity. However, the effect on body weight, clinical symptoms and toxic symptoms has not been studied yet. **Objectives:** Accordingly, the objective of this research was to determine the effect of *Arcangelisia flava Merr.* ethanol extract obtained from gradual extraction method on parameters of body weight, clinical symptoms and toxic symptoms in male mice. **Material and Methods:** A total of 16 male mice aged 6-8 weeks weighing 20-40 grams were divided into 4 groups. The control group was only given 1% sodium-carboxymethyl cellulose (CMC-Na), while the treatment group was given *Arcangelisia flava Merr.* ethanol extract at doses of 800, 900, and 1000 mg/kg BW orally for 28 days. At week 1 to week 4, body weight, clinical symptoms and toxic symptoms were observed for male mice. **Result:** Observations of toxic symptoms and symptoms showed that there were several symptoms that appeared in male mice and changes in body weight. There were no significant differences from t0 to t1, t0 to t2, t0 to t3, and t0 to t4 for each treatment ($p>0,05$). **Conclusions:** The administration of *Arcangelisia flava Merr.* ethanol extract at doses of 800, 900, and 1000 mg/kg BW for 28 days did not affect weight gain or loss, but these extracts have an effect on symptoms in test animals such as straight fur and strange behavior.

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ABSTRAK

Latar Belakang: Kayu kuning (*Arcangelisia flava* Merr.) salah satu tanaman asli Indonesia yang mengandung alkaloid, fenolik, flavonoid, saponin, tanin dan berberine. Tanaman ini telah terbukti bahwa memiliki aktivitas sebagai antimikroba, antioksidan, antihiperlipidemia, dan antikanker, namun belum diketahui pengaruhnya terhadap berat badan, gejala klinis dan gejala toksik pada hewan coba.Tujuan: Penelitian ini bertujuan mengetahui pengaruh ekstrak etanol *Arcangelisia flava Merr.* dengan metode maserasi bertingkat terhadap parameter berat badan, gejala klinis dan gejala toksik pada mencit jantan. Bahan dan Metode: Sebanyak 16 ekor mencit jantan berusia 6-8 minggu dengan bobot 20-40 gram dibagi menjadi empat kelompok. Kelompok kontrol hanya diberi sodium-carboxymethyl cellulose (CMC-Na), sedangkan kelompok perlakuan diberi ekstrak *Arcangelisia flava Merr.* dosis 800, 900, dan 1000 mg/kg BW per oral selama 28 hari. Pada minggu ke 1 hingga minggu ke 4, dilakukan pengamatan berat badan, gejala klinis dan gejala toksik terhadap mencit jantan. Hasil: Hasil pengamatan gejala klinis dan gejala toksik menunjukan adanya beberapa gejala yang muncul pada mencit jantan dan perubahan berat badan. Tidak terdapat perbedaan yang nyata dari t0 ke t1, t0 ke t2, t0 ke t3, dan t0 ke t4 untuk masing-masing perlakuan ($p>0,05$). Kesimpulan: Pemberian ekstrak *Arcangelisia flava Merr.* dosis 800, 900, dan 1000 mg/kg BB selama 28 hari tidak mempengaruhi kenaikan atau penurunan berat badan, namun ekstrak tersebut berpengaruh terhadap gejala pada hewan uji seperti bulu berdiri dan tingkah laku aneh.

Kata kunci: Uji Toksisitas, Subkronis 28 hari, Maserasi Bertingkat, Ekstrak *Arcangelisia flava* Merr.

INTRODUCTION

Arcangelisia flava Merr. or yellow root plant is a native Indonesian plant that can be used as herbal medicine to treat jaundice, digestion, intestinal worms, tonic, fever, menstrual laxative, and cancer sores (Kaharap dkk., 2016). Based on research that have been carried out by several researchers, yellow root has been shown to have antimicrobial, antioxidant, antihyperlipidemic, and anticancer activities. The content of secondary metabolites that have been studied by Sari *et al.*, (2018) proves that there are alkaloids, phenolics, flavonoids, saponins, tannins and berberine. Considering the various uses of yellow root but the usage is still based on hereditary experience or empirically, it is still necessary to carry out further research so that it can become new scientific information regarding the efficacy, side effects and toxic effects. Therefore, to determine the safety level of yellow root extract, it is necessary to carry out a toxicity test. The safety of a plant extract can be determined through several toxicity test methods such as acute, subchronic, chronic toxicity tests and specific tests. Subchronic toxicity tests were carried out on herbal products given in repeated doses for 28 or 90 days. Research from Rachmawati and Ulfa (2018), proved that the subchronic toxicity test of yellow root methanol extract with the maceration method given orally at doses of 250, 500, and 750 mg/kg BW within 28 days on male mice do no affect the liver and the renal, but caused congestion of liver blood vessel, but the liver cell did not experience necrosis. Based on this, a subchronic toxicity test of yellow root ethanol extract obtained from gradual maceration method with doses of 800, 900, and 1000 mg/kg BW orally within 28 days that aims to determine the effect of yellow root ethanol extract is carried out.

MATERIAL AND METHODS

Materials

This research used an experimental research design with a completely r1andomized design (CRD), in which there were 4 groups with completely randomized treatment. There was 1 control group given CMC-Na, and 3 groups given yellow root extract at doses of 800, 900, and 1000 mg/kg BW orally for 28 days. The materials used were yellow root (*Arcangelisia flava* Merr.). The root part of the plant was obtained from Pontianak City, West Kalimantan. CMC-Na, n-hexane, ethyl acetate, ethanol 96%, standard feed, and aquadest.

Methods

The subchronic toxicity test in this research was carried out at the Natural Materials Laboratory and Pharmacology Laboratory, Pharmacy Study Program, Institute of Health and Science Technology, RS dr. Soepraoen Malang, in January-May 2022. Ethical test has been carried out with the number No.837/eC/KEPK-FKIK/2022. A total of 500 grams of yellow root were ground to obtain simplicia powder which was then macerated with hexane solvent for 3 days, the resulting filtrate was then filtered using a glass funnel and mori cloth then the residue obtained was re-macerated in the same way but with ethyl acetate solvent, then the residue was repressed. the third time with 96% ethanol solvent. The resulting filtrate was concentrated with a rotary evaporator to obtain a thick extract of yellow root.

Subchronic toxicity test was carried out on 16 male Wistar mice aged 6-8 weeks with a weight of 20-40 grams. Before treatment, mice were acclimatized for 1 week and given standard feed and drinking water. Then, 16 mice were randomly divided into 4 treatment groups. In the control group, mice were only given CMC-Na while the 3 test groups were given the extract of *Arcangelisia flava Merr.* doses of 800, 900, and 1000 mg/kg BW orally for 28 days. Observational data were analyzed using SPSS version 16 the statistical program Shapiro-Wilk Test normality test and continued with Paired Sample T-Test and Wilcoxon Test alternative.

RESULTS AND DISCUSSION

Extraction of yellow root using gradual maceration method with the first solvent is hexane (non-polar), the second solvent is ethyl acetate (semi-polar), and the third solvent is ethanol (polar). The results of the extraction can be seen in Table 1.

Table 1. Result of extraction yeild

No	Simplicia Weight (g)	Solvent	Extract Weight (g)	Yeild (%)
1.	500	Hexane Solvent	0,2	0,04
2.	500	Ethyl Acetate Solvent	5,4	1,08
3.	500	96% Ethanol Solvent	32	6,4

The subchronic toxicity test in this research used the gradual maceration method with the aim of obtaining extracts with specific compounds in each solvent, so as to produce purer and more abundant extracts. The results of the extraction using the gradual maceration method obtained a yield of 6,4% of ethanol solvent extract. Ethanol solvent is one of the most optimal solvents in obtaining total flavonoid levels (Riwanti *et al*., 2020). According to Nurhayati *et al.* (2009) and Dewatisari *et al.* (2018), the high yield value indicates the amount of bioactive content contained in the plant. The yield value of more than 10% can be said to be good. The higher the yield value of the extract, the higher the content of substances that can be attracted to a raw material (Budiyanto, 2015). The low yield value of ethanol extract (6,4%) might be because the bioactive compounds contained in plants has been taken up by previous solvents. The extract that has been obtained from the extraction is then carried out for organoleptic examination. Types of organoleptic examination consist of color, taste, smell, shape and homogeneity (Table 2).

Table 2. Result of yellow root ethanol extract organoleptic observation

No	Type	Result	Result of Ratnasari and Handayani, 2018
1.	Color	Yellow to Brownish	Yellow to Brownish
2.	Taste	Bitter	Bitter
3.	Smell	Typical of Yellow Root	Typical of Yellow Root
4.	Shape	Thick Extract	Liquid
5.	Homogeneity	Homogeneous with CMC	Homogeneous

From the results of the organoleptic examination, the ethanol extract of yellow root has a brownish-yellow color, a bitter taste, a distinctive smell of yellow root, and a thick and homogeneous extract with respect to CMC-Na. The inspection shows that the results of the examination are in accordance with the examination by Ratnasari and Handayani (2018).

The results of observations for 28 days with the administration of the test material found that at doses of 900 mg/kg BW and 1000 mg/kg BW have caused several toxic symptoms, in the form of straight fur or so-called piloerections that showing adrenergic or adrenaline effects (Loomis, 1978). The sympathetic nervous system is a nerve involved in activities related to the body's energy expenditure so that it can increase blood flow to the muscles, causing epinephrine secretion, causing an increase in heart rate and blood sugar levels due to the work of the sympathetic nervous system during periods of increased activity (Tjay and Kirana, 2001). Toxic symptoms experienced by mice are strange behavior in the form of silence. Normally, mice will move to and for sometimes, it making spontaneous movements by walking fast or running at high speed which indicates stimulation of the CNS or ganglia or neuromuscular junction. On the other hand, if the mice are silent until they fall asleep, it shows depression of the central nervous system, and when touched there is no reaction, indicating anesthesia

(Pudjiastuti, 2009). Meanwhile, at a dose of 800 mg/kg BW, it did not show any toxic symptoms in test animals.

Table 3. Average of symptom observation

Group	Average Number of Symptoms in Weeks				
	0	Week 1	Week 2	Week 3	Week 4
CMC-Na	-	-	-	-	-
800 mg/kg BW	-	-	-	-	-
900 mg/kg BW	-	1	-	-	-
1000 mg/kg BW	-	2	1	-	-

The results of the observation of these symptoms resulted in the response given from each test animal at different dose groups. This is a natural thing due to differences in the physiological conditions of mice such as body weight, age, and metabolic processes so that these factors will affect some of the parameters measured (Wood, 1999). Of the three doses given, there was no mortality rate, only a few toxic symptoms such as straight fur and strange behavior in the form of bending over. In the 1st week of the dose of 900 mg/kg BW of yellow wood extract, there was one mouse that had straight fur. Meanwhile, at dose 1000 mg/kg BW yellow wood extract, two mouse experienced straight fur in the first week and one mouse experienced strange behavior in the second week (Table 3). Observations of these symptoms experienced the differences between the control group and the test group. The symptom observation data in this research were examined for normality assumptions using the Shapiro-Wilk Test. In the symptom observation data generated from the Shapiro-Wilk Test, the data stated that the significance value was 0,000 ($p<0.05$) using 95% confidence level. Therefore, the data is not normally distributed and the hypothesis test is included in non-parametric statistics. Therefore, the Wilcoxon test can be further carried out.

From the analysis produced by the Wilcoxon Test, it is found the significance value is 0,000 ($p<0.05$) using a significance level of α 0,05. (Therefore, the hypothesis is rejected that) means there are differences in symptoms between the control group and the test group, so it can be concluded that there is an effect of giving yellow root ethanol extract on symptoms in male mice.

In addition to observe the symptoms, there are observing body weight during the treatment to find out whether there is a change in the body weight of the test animals between before and after administration of yellow root extract. The data obtained the averaged body weight at weeks 1-4 and a graph was made to see the changes during the research.

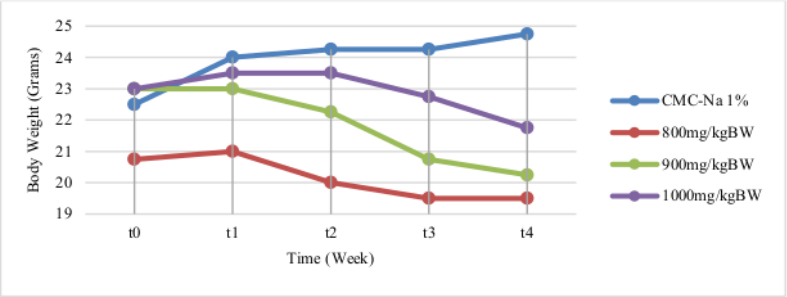

Figure 1. Average line diagram of body weight observation

It can be seen that there was an increase in body weight of mice in the first week (t0-t1) for all treatments. However, in the second week (t1-t2) until the fourth week (t3-t4) after the treatment, the weight of the mice decreased due to adaptation to the environment and biological conditions of the mice. In the control group, no extract was given, except CMC-Na, so there was no weight loss in mice. In contrast to the control group, the group given the yellow wood ethanol extract at different doses experienced weight loss due to reduced appetite (Figure 1). According to Sireeratawong *et al* (2016), experimental animals that received high doses would lose weight due to decreased appetite. During the treatment, the test animals were still given a carbohydrate source in the form of sweet corn as food to maintain body weight during the treatment, the feeding did not affect during the research process because each test animal treatment would be fasted first for ± 18 hours but were still given water.

The body weight observation data in this research were examined for normality assumptions using the Shapiro-Wilk Test in each group and continued by either by paired samples T-test or wilcoxon test (Table 4).

Table 4. Average of body weight observation

Group	Average Body Weight (Grams)					p value (α 0,05)		
	t0	t1	t2	t3	t4	Shapiro-Wilk Test	Paired sample T-test	Wilcoxon Test
CMC-Na	22,5	24	24,25	24,25	24,75	0,002*		0,197
800mg/kgBW	20,75	21	20	19,5	19,5	0,653	0,080	
900mg/kgBW	23	23	22,25	20,75	20,25	0,018*		0,102
1000mg/kgBW	23	23,5	23,5	22,75	21,75	0,085	0,431	

In the symptom observation data generated from the Shapiro-Wilk Test, the data of CMC-Na and doses of 900 mg/kg BW found that the significance value was 0,002 and 0,018 ($p<0,05$) using α 0,05 level. Therefore, the data is not normally distributed and the hypothesis test is included in non-parametric statistics. Therefore, the Wilcoxon Test can be carried out. Meanhile, the data of doses of 800 and 1000

mg/kg BW found the p value were 0,653 and 0,085 (p>0,05) means that the data is normally distributed and the hypothesis test is included in parametric statistics. Therefore, the paired samples T-test can be carried out. Further analysis by the Wilcoxon test and paired samples T-test, it was found that the significance value of all treatments was >0,05 (The hypothesis was accepted) which means that there was no significant difference between initial body weight and body weight at week 4. Therefore, it can be concluded that there is no effect of changes in body weight on the administration of CMC- Na and all doses of treatments.

It is necessary to conduct an oral subchronic research for 90 days to find out more toxic symptoms and clinical symptoms in male mice and histopathology to determine the condition of vital organs in mice and perform SGOT and SGPT tests using blood serum of test animals to obtain more complete information and accurate regarding organ damage due to exposure of the extract of *Arcangelisia flava* Merr.

CONCLUSION

Subchronic oral administration of yellow root ethanol extract (*Arcangelisia flava Merr.*) for 28 days with doses of 800 mg/kg BW, 900 mg/kg BW, and 1000 mg/kg BW caused a toxic effect on mice, which caused symptoms such as straight fur and strange behavior but did not affect body weight gain or loss.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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