

ACUTE TOXICITY TEST OF COCOA SEED METHANOL EXTRACT MOUTHWASH AGAINST MICE

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ABSTRACT

Indonesia is famous for its natural wealth of medicinal plants. Cocoa plant (*Theobroma cacao* L.) is one of the herbal medicinal plants which is used as an alternative to cure diseases. Cocoa beans contain flavonoids, saponins and tannins which can inhibit the growth of bacteria and have potential as antioxidants and anti-bacterial. The calculation of the toxic test really needs to be taken into account in the experiment. Toxicity test is important to do to estimate the level of damage caused by a compound to biological and non-biological materials. Toxicity tests are generally used to determine unwanted effects of a drug, especially on the incidence of cancer, heart, liver and skin disorders or eye irritation. This study aims to determine the concentration of acute toxicity, safety level, cocoa bean mouthwash and the clinical symptoms it causes. The research method is a laboratory experiment based on the guidelines of the Organization for Economic Co-operation and Development (OECD). It uses minimal animal models, and testing time is relatively fast. A total of 12 female mice aged 6-8 weeks weighing about 25 g were divided into 4 treatments (n=4). Group 1 was given a dose of 1 mg/g BW, group 2 was given a dose of 2 mg/g, group 3 was given a dose of 3 mg/g and group 4 (control) was given 2 mL/kg BW of water. The extract was given orally using a sponge every day for 14 days. Research result; Based on the dose and death groups of mice after administration of methanol extract of Laban leaves, no dead mice were found either in the 1 mg/bb kg, 2 mg/bb kg dose group, the 3 mg/bb kg dose group, and the aquadest water control group. All 12 mice were alive. Based on the treatment dose group, the administration of methanol extract of Laban leaves after 24 hours and from the first day to the 14th day did not show symptoms of standing hair, incoordination of nerves, and did not show symptoms of depression, which meant that there were no symptoms of toxicity in mice. Based on body weight, each group showed an increase in body weight, the concentration given did not significantly affect body weight. Conclusion; Variation of the concentration of methanol extract mouthwash of cocoa beans was not toxic, did not cause death of rats, did not cause toxic symptoms and did not affect the body weight of rats. It is recommended to use methanol extract of cocoa beans as a basic ingredient for making mouthwash ingredients.

Keywords: Toxicity, mouthwash, Cocoa Beans

1. INTRODUCTION

Cocoa beans have several active components, one of which is polyphenols, the polyphenol content in cocoa beans is dominated by catechins and epilagalocatechins, anthocyanins, and procyanidins. Polyphenols have preventive activity against infectious and degenerative diseases including oral diseases (Nuraskin et al., 2019). Polyphenols can reduce the formation of biofilms and acid products from *S. mutant* which is an antibacterial compound that

can inhibit the growth of *S. mutant* which is the main bacterium that causes dental caries (Chassagne et al., 2016).

Toxicity testing is important to do to estimate the damage caused by a compound to biological or non-biological substances. (Nuraskin et al., 2021) Toxicity testing generally aims to determine the unwanted effects of a drug. (Sasmito et al., 2015)

In general, the use of traditional medicines for treatment is of great interest to the public, but

the doses used are not in accordance with the official standard usual doses and maximum doses. This is what allows side effects to occur due to the use of traditional medicines that exceed standard doses or usual doses. Therefore, it is necessary to conduct a toxicity test on a drug product that will be marketed. Methanol extract of cocoa beans has never been tested for toxicity.

The toxicity testing method was chosen based on the Organization for Economic Co-operation and Development (OECD) guidelines. This method is a standard method that is recognized by 33 European countries that are members of the OECD. The main advantages of this method are the minimal use of animal models (mouse) and the relatively fast testing time. A total of 12 female mice aged 6-8 weeks with body weight around 25 g were divided into 4 treatments (n = 4) and were used for clinical trials. Group 1 was given the extract at a dose of 1 mg/kg bw, group 2 at a dose of 2 mg/kg, group 3 at a dose of 3 mg/kg, and group 4 (control) was given distilled water 2 mL/kg body weight. The extract was given orally using a sponge every day for 14 days. Clinical observations, depressive symptoms, standing hair were observed, and deaths occurring during the test period were observed. (Sasmito et al., 2015)

Research objectives are;

This study aims to determine the concentration of acute toxicity, safety level, cocoa bean mouthwash and the clinical symptoms it causes.

RESEARCH METHODS

His research was conducted at the Pharmacology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh since March 2022. The samples for this study were 12 female mice obtained from the Pharmacology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University. Meanwhile, the test material used was methanol extract mouthwash of cocoa beans. The equipment used was a mouse cage,

a drinking bowl, a syringe, an oral probe, a beaker, a measuring cup, a pipette, a pestle and an animal scale. The materials used were cocoa seed methanol extract mouthwash, distilled water, mice food.

The research procedure is; using Organization for Economic Co-operation and Development (OECD) guideline 423 (2002). This method is a standard method recognized by 33 European countries as members of the OECD. The advantages of this method are the small use of animal models (mice) and the relatively fast test time (Sasmito, et al., 2015). First prepared 12 female mice aged 6-8 weeks with body weight around 25 g, divided into 4 treatments (n = 4 groups) each group consisting of 3 mice. Adaptation 5 days before the intervention, Weigh the body weight, fast 2 hours before the intervention, give the extract. Group 1 is given the extract at a dose of 1 mg/kg body weight, group 2 at a dose of 2 mg/kg, group 3 at a dose of 3 mg/kg, and group 4 (control) was given 2 mL of distilled water/kg body weight. Extract administration was carried out orally using a spet containing cocoa bean methanol extract every day for 14 days of clinical observation after 24 hours of clinical observation, symptoms of depression, standing hair were observed, and deaths that occurred during the test period were observed.

RESULTS

The acute toxicity test in this study aims to assess the safety of the drug in terms of the toxicity of the drug when used. Three levels of cocoa bean extract dose used were 1 mg/kg BW, 2 mg/kg BW, 3 mg/kg BW, and 2 mg/kg BW aquadest.⁸ The test preparations were given once a day for 14 days and the research data were measured 24 hours after the administration of the test preparations. The toxicity of cocoa bean extract was measured by the number of dead test animals, symptoms of depression, hair standing, and symptoms of nervous incoordination.

Extra ct Dosage Group	N	WEIGHT (grams)/day									
		H1	H3	H5	H7	H9	H11	H13	H15		
Aquad	1	25	25	25	26	26	27	28	28		
	2	26	26	26	26	27	27	27	27		
	3	25	26	26	26	26	27	28	28		
Ekstrak 1 mg	1	26	25	26	26	26	27	27	28		
	2	26	26	26	26	27	27	27	28		
	3	25	26	26	26	27	27	27	27		
Ekstrak 2 mg	1	26	26	26	27	27	27	28	28		
	2	25	25	26	26	26	27	28	28	1	1
	3	26	26	26	26	26	27	27	27	3	4
Ekstrak 3 mg	1	25	25	25	26	26	27	28	28	0	0
	2	25	25	26	26	27	28	28	28	0	0
	3	25	25	26	26	27	27	27	27	0	0
2 mg g BB	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0
3 mg g BB	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0
2 ml aquades	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0

Table 1. Mice mortality for 14 days after administration of cocoa bean extract mouthwash

Based on table 1, the dose and mortality group of mice after administration of cocoa bean extract at a dose of 1g/bb kg, a dose group of 2g/bb kg and a group at a dose of 3 g/bb kg and a control group of 2 ml distilled water, there were no mice that died well. All 12 mice were alive.

Table 2. Toxicity of mice for 14 days after giving the cocoa bean extract mouthwash

Based on table 2, we can see, all the test animals in each treatment dose group of laban leaf methanol extract after 24 hours and the first day until the 14th day did not show symptoms of standing hair, incoordination of nerves, symptoms of depression, which means no symptoms were found. toxicity in mice.

Table .3. Dosage group of cocoa bean extract mouthwash based on body weight of mice

Extra ct Dosa ge Group	Trea tme nt	Hair standing, Nerve incoordination, Symptoms of depression dosing in /day														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1 mg	1mg/g BB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 mg	2mg/g BB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3m g	3mg/g BB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 ml aquades	2 ml air	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Based on table 3, we can see that all experimental animals in each group showed increased body weight. Which means the higher the dose given and the longer the dose given does not affect body weight. Normal body weight which means no toxicity based on the weight of the mice.

DISCUSSION

The acute toxicity test in this study aims to assess the safety of cocoa bean extract. The three dose levels used were 1 mg/kg, 2 mg/kg, 53 mg/kg, 2 ml of distilled water, group. The test preparations were given once a day and the research data were measured every day 24 hours after the test preparations were administered. Toxicity was measured from the number of dead test animals, clinical signs and symptoms of poisoning, changes in body weight.

Results Based on the dose and death groups of mice after administration of methanol extract mouthwash of cocoa beans, there were no dead mice in the 1mg/bb kg dose group, the 2mg/bb kg dose group and the 3 mg/bb kg dose group, and the aquadest water control group . All 12 mice were alive.

Based on the observation of the groups of mice toxicity symptoms, it can be seen that all test animals in each treatment dose group of cocoa bean methanol extract mouthwash after 24 hours and from the first day to the 14th day did not show symptoms of standing hair, incoordination nerves, symptoms of depression, which This means that there were no signs of toxicity in mice.

Based on the dose group Administering cocoa bean methanol extract mouthwash Based on body weight of mice, it can be seen that all experimental animals in each group showed an increase in body weight. Which means the higher the dose given and the longer the dose given affects body weight significantly. Normal body weight which means no toxicity based on the body weight of the mice. Administration of methanol extract of cocoa beans did not interfere with the health and appetite of the mice. This can be seen from the growth of the rats' body weight for 14 days. All mice in each treatment group experienced a significant increase in body weight.

In this study using a volume concentration of 1% (1 mg), 2%, (2 mg) 3% (3mg) in accordance with the provisions of the use of therapeutic materials. According to (Anderson T.Hara, 2017) The therapeutic ingredients used should be 0-2%, there are several active ingredients that have therapeutic functions for dental and oral health, including: Fluoride, densifying agents, anti-calculus agents, anti-tartar agents , antimicrobial agent. Therapeutic materials used according to SNI standards are 0-2%, namely: fluoride, desensitizing agents, anti-tartar agents and antimicrobial agents. The standard therapeutic agent for children is 0.7 mg, youth is 1.5 mg, and the standard for adults and pregnant women is 1.5 mg to 3.0 mg (Amount, 2012).

Based on the provisions of Balai Pom, 1000-500 mg of extract is toxic, 50-500 mg is moderately toxic, 500-5000 is mildly toxic and 5-15 grams is practically non-toxic. With this in mind, using concentrations of 1 gram, 2 grams, 3 grams is not dangerous, so the test dose does not need to be continued by using a higher dose of the test material(Amir, 2014).

If the 5 gram extract used is toxic, it must be stopped. Determination of toxic doses and lethal doses using the OECD method has advantages over other toxicity testing methods because it uses a small number of experimental animals and easy testing techniques. Regulations for toxicity testing with experimental animals must pay attention to animal welfare, by using as few as possible the number of experimental animals, and the results of the tests will be useful for application to humans.(Saryanto, 2015).

Toxicity testing generally uses at least 3 doses (low, medium and high) and uses a control group to compare the effects of the treatment groups. (Sinensis et al., 2009). In vivo toxicity tests are still needed because they have several advantages, including data related to the condition. normal physiology and biochemistry and the results of in vivo testing in experimental animals can be interpolated to humans or as a predictor of toxicology ((Hafidhah et al., 2017).

CONCLUSION

Acute toxicity test results

The administration of methanol extract mouthwash of cocoa beans was carried out orally using a spet sonde every day for 14 days of clinical observation after 24 hours. Clinical observations, all concentration variations did not cause death of mice, did not cause symptoms of poisoning, and did not affect body weight of mice after mouthwash administration methanol extract of cocoa beans.

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