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Acute Oral Toxicity Test of Antihypertensive Polyherbal Preparations Containing *Allium sativum Curcuma aeruginosa* & *Amomi fructus*

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ABSTRACT

Hypertension is a major factor causing atherosclerotic cardiovascular disease, heart failure, stroke, and kidney failure. Polyherbal preparations containing garlic (*Aliium sativum*), temu ireng (*Curcumae aeruginosae*) and cardamom (*Amomi fructus*) have been widely used to treat hypertension. Despite widely used in community, its safety has not been evaluated. This study aimed to evaluate the single dose oral safety of the polyherbal. The acute oral toxicity test was done using fixed dose methods. The clinical examination was done after administration of the polyherbal and continued until the 14th day to check for symptoms of toxicity, and changes in body weight. On day 15, the animal was sacrificed and histopathological examination was conducted. The body weight did not differ between groups. However, there was an increase in body weight in a group that received polyherbal at a dose of 2000 mg/kg. The absolute and relative organs weight was also similar among groups. There were no macroscopic and histopathological changes in vital organ. The polyherbal preparations containing Aliium sativum, Curcumae aeruginosae and Amomi fructus is safe with LD_{50} >2000-5000 mg/kg in Wistar rats.

Keywords*: acute oral toxicity test; Aliium sativum; Curcumae aeruginosa; Amomi fructus.*

INTRODUCTION

Hypertension is a major factor causing atherosclerotic cardiovascular disease, heart failure, stroke, and kidney failure (Smeeltzer & Bare, 2013). Comprehensive management of hypertension includes regulation of diet, exercise, and administration of antihypertensive drugs. Some medicinal plants that grow in Indonesia have been used traditionally by the community to treat hypertension (Sholikhah *et al.,* 2020). One of several herbal medicines used for hypertension in Indonesia is polyherbal preparations containing garlic (*Aliium sativum*), temu ireng (*Curcumae aeruginosae*) and cardamom (*Amomi fructus*) extracts.

Garlic (*Aliium sativum*) has been widely used to treat various cardiovascular problems. Studies showed that garlic can lower blood pressure (Ried *et al*., 2016; Wang *et al*., 2015). Oxidative stress is a major mechanism in endothelial dysfunction and vascular damage, which has been shown to play an important role in the pathogenesis of hypertension (Baradaran *et al*., 2014; Guzik &Touyz, 2017). Temu ireng (*Curcumae aeruginosae*) and cardamom (*Amomi fructus*) have antioxidant activity (Choudhury *et al*., 2013; Nurcholis *et al*., 2015).

Polyherbal preparation containing garlic (Aliium sativum), temu ireng (Curcumae aeruginosae) and cardamom (Amomi fructus) have been used for hypertension. However, its safety has not been evaluated. Toxicity tests must be carried out to evaluate the general safety of a compound as a whole in test animals. Acute toxicity test is the administration of a single dose to measure the degree of toxic effect of a compound that occurs within a short time (BPOM, 2022). Therefore this study aimed to evaluate the safety of polyherbal oral administration by acute toxicity test.

METHOD

The research was conducted at the Pharmacology Laboratory, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah

Mada. This research has obtained permission from the Medical and Health Research Ethics Commission, Faculty of Medicine, Universitas Gadjah Mada based on a certificate of ethical feasibility with the number KE/FK/0824/EC/2022.

Polyherbal Formulations

The polyherbal formulation was obtained from PT. Swayasa Prakarsa, Yogyakarta, Indonesia. Each capsule of the polyherbal formulation weighed 400 mg containing 240 mg of *Allium sativum* tuber powder, 60 mg of *Curcuma aeruginosa* rhizome powder, 45 mg of *Amomum fructus* fruit powder, and 55 mg of excipients.

Oral Acute Toxicity Test

A total of 13 female Wistar rats aged 8-12 weeks with body weight ranging from 200 to 300 grams. Acute oral toxicity tests were performed according to OECD 420 guidelines using a fixed dose procedure. The test is divided into 2 stage, initial and main stage. The initial stage is carried out to find a suitable initial dose for the main test. Initial doses of 5, 50, 300 and 2000 mg/kg. The main stage was carried out at the dose level at which death occurred in the preliminary test or the highest dose reached in initial stage, dose 2000 mg/kg. In this main test, 5 test animals are required on the test dose. The five animals consisted of 1 animal from the preliminary test and 4 additional animals.

Observations after single administration of the polyherbal were made for 30 minutes, 4 hours and 8 hours for the first 24 hours and once a day for 14 days. The observation result was compared to the control group. Body weight was measured every week. Absolute organ weight and relative organ weight were also measured after the animal sacrificed on day 15. After sacrificed, the organ was analyzed macroscopically and microscopically.

Data analysis

Statistical analysis was conducted using SPSS version 25. The normality of the data was checked using the Shapiro-Wilk test. Paired T-test was performed to analyze paired data before and after the intervention. Data are presented in mean ± standard deviation (SD) VBN.

RESULTS AND DISCUSSION

Results

All rats received single dose of polyherbal preparations did not show any toxic symptoms on 30 minutes, 4 hours and 8 hours, 24 hours and everyday until 14 days after polyherbal preparation administration. The Initial stage start with administration of polyherbal preparation at 5 mg/kg. Both polyherbal treated rat and control rat did not show any toxicity symptoms. Thus, the initial stage continued on dose 50 mg/kg, 300 mg/kg, and 2000 mg/kg. Even after administration of polyherbal preparation on the highest dose in the protocol, the rat still not showing any signs of toxicity. Therefore, the main stage was done on dose 2000 mg/kg. In the main stage, four rats were added to complete sample number required in the main study. None of the rats in the main study showed toxicity signs. Table I shows the observation of toxicity signs.

Observation of changes in body weight was carried out by weighing the weight of the test animals before and after administration of polyherbal preparation (day 7 and day 14). The body weight data are shown in Table II. There was no significant difference in the mean of weight at before, day 7 and day 14 in the control group ($p = 0.255$). However, in the 2000 mg/kg group, the mean of weight at before, day 7 and day 14 was different ($p=0.001$).

The weight gain is similar between control group and dose 2000mg/kg group. Table III shows the weight gain on the 7th and 14th days in the control group and 2000 mg/kg group.

The absolute organ weight, including heart, liver, lungs, kidney, spleen, and ovary, was not different between control group and dose 2000 mg/kg group (Table IV).

The relative organ weight, including heart, liver, lungs, kidney, spleen, and ovary, was not different between control group and dose 2000 mg/kg group (Table V).

The macroscopical examination of organs, heart, liver, lungs, kidney, spleen, and ovary showed no pathological sign (Table VI).

The results of histopathological examination showed no changes of the heart, liver, lungs, kidneys, spleen, and ovary in the control and treatment groups, as shown in Figure 1.

Table I. Results of Observation of Toxic Signs on Test Animals

Group	Number of mice	Toxic Symptoms Appear				
		30 minutes	4 hours	8 hours	24 hours	14 days
Preliminary Test						
Control	4	-	-	-	-	-
Dosage 5 mg/kg	1	-	-	-	-	-
Dosage 50 mg/kg	1	-	-	-	-	-
Dosage 300 mg/kg	1	-	-	-	-	-
Dosage 2000 mg/kg	1	-	-	-	-	-
Main Test						
Control	1	-	-	-	-	-
Dosage 2000 mg/kg	4	-	-	-	-	-

Description: (√) exists; (-) does not exist

Table II. Body Weight Before and After Administration to Test Animals

Group	No. Rat	Body weight (grams)			P value (between before, day 7, day 14)
		Before	7th day	14th day	
Control	1	207	201	201	0.255
	2	187	190	193	
	3	196	201	207	
	4	204	202	203	
	5	209	220	232	
	Mean±SD	200.6±9.1	202.8±10.8	207.2±14.8	
2000 mg/kg	1	204	210	214	0.001
	2	204	216	220	
	3	181	190	203	
	4	203	207	212	
	5	189	195	198	
	Mean±SD	196.2±10.6	203.6±10.8	209.4±8.8	
P value (between control and 2000 mg/kg)		0.501	0.901	0.782	

Table III. Weight Gain in Test Animals

Group	No. Rat	Body weight (grams)		P value (between before, day 7, day 14)
		7th day	14th day	
Control	1	0.00	-2.90	0.184
	2	1.55	1.60	
	3	2.90	2.55	
	4	0.49	-0.98	
	5	5.17	5.26	
		2.02±208	1.11±3.16	
2000 mg/kg	1	1.87	2.86	0.411
	2	1.82	5.56	
	3	6.40	4.74	
	4	2.36	1.93	
	5	1.52	3.08	
		2.79±2.04	3.63±1.48	
P value (between control and 2000 mg/kg)		0.144	0.570	

Table IV. Data on Absolute Organ Weight after Administration of Polyherbal Dosage of 2000 mg/kg and Control

Group	Absolute Organ Weight of Test Animal (X ± SD)					
	Heart	Liver	Lungs	Kidney	spleen	Ovary
Control	0.678±0.026	7,644±1,236	1,230±0.156	0.738±0.031	0.772±0.034	0.692±0.144
2000 mg/kg	0.664±0.068	6,012±1,247	1.318±0.235	0.855±0.302	0.728±0.094	0.588±0.123
P value (between control and 2000 mg/kg)	0.680	0.071	0.505	1.000	0.355	0.254

Table V. Relative Organ Weight Data After Administration of Polyherbal Dosage of 2000 mg/kg and Control

Group	Absolute Organ Weight of Test Animal (X ± SD)					
	Heart	Liver	Lungs	Kidney	spleen	Ovary
Control	0.329±0.028	3.724±0.741	0.593 ±0.055	0.375±0.018	0.374±0.033	0.333±0.033
2000 mg/kg	0.319±0.046	2.870± 0.568	0.631±0.121	0.408±0.141	0.348±0.041	0.283±0.071
P value (between control and 2000 mg/kg)	0.697	0.075	0.542	0.602	0.295	0.265

Table VI. Macroscopic Examination of Test Animals After Necropsy

Organ	Observation result
Heart	Smooth surface, flat, brownish red
Liver	Smooth surface, flat, pointed flat tip, brownish red
Lungs	Smooth surface, flat, no nodules, pink
Kidney	Smooth surface, flat, brownish red
Spleen	The surface is flat, flat, smooth, red-brown in color
Ovary	Smooth surface, white color

The results of histopathological scoring of the kidneys are ordinal data. Furthermore, to facilitate the analysis, the data is converted into interval data using the method of successive interval (MSI). The results of renal histopathology scoring showed that there was no significant difference between the control group and the 2000 mg/kg polyherbal preparation (p = 0.729) this is shown in table VII.

Discussion

Polyherbal preparations containing garlic (*Aliium sativum),* temu ireng (*Curcumae aeruginosae*) and cardamom (*Amomi fructus*) in the acute toxicity test showed no clinical signs of toxicity and death of the test animals at 24 hours after administration until day 14 of observation. The LD_{50} of the polyherbal preparation was > 2000 mg/kg based on acute oral toxicity test in rat. Research conducted in rat by Lawal *et al* (2016) showed that aqueous extract of garlic (*Aliium sativum*) at a dose of 5,000 mg/kg showed clinical signs of toxicity in the form of tachycardia and disorientation but no deaths were recorded so that the LD_{50} value was > 5,000 mg/kg. In a single dose of cardamom extract, LD_{50} > 2000 mg/kg in rat was obtained (Yudhani *et al.,* 2019, Marjawan, 2022). Chloroform extract and methanol extract of temu ireng (*Curcumae aeruginosae*) in mice obtained LD_{50} value of 3.03 g/kg. On oral administration of

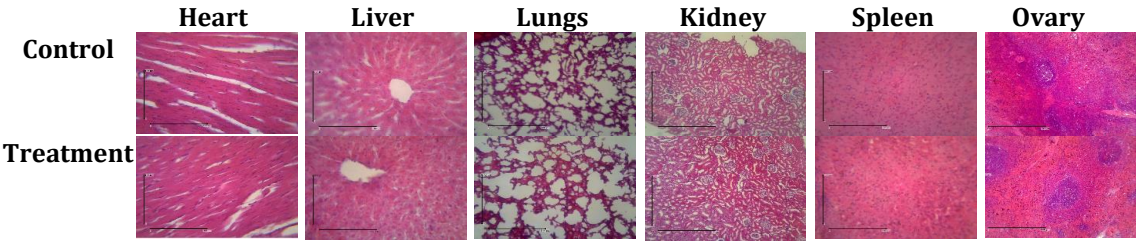

Figure 1. Effect of 2000 mg/kg polyherbal preparation on various histopathology of rat organs in acute oral toxicity test (hematoxylin and eosin staining, 100x magnification)

Table VII. Average Data of Kidney Histopathology Score

Group	Average renal histopathology score (Mean±SD)
Control	2.55±0.31
2000 mg/kg	2.63±0.20
P value (between control & 2000 mg/Kg)	0.729

temu ireng water extract (*Curcumae aeruginosae*) no dead were found in mice at a dose of 10 g/kg (Reanmongkol *et al.,* 2006). Polyherbal preparations containing garlic (*Aliium sativum*), temu ireng (*Curcumae aeruginosae*) and cardamom (*Amomi fructus*) did not cause toxic effects in the acute toxicity test with an LD value of $_{50}$ > 2000 mg/kg.

The changes in body weight of the test animals are used to evaluate the general health condition of the test animals as a sign of the presence or absence of toxicity. There was no significant difference in the weight of the test animals in the control group before treatment, on the 7th and 14th days. In the 2000 mg/kg group, the body weight was significantly different between before treatment, on the 7th and 14th days. Purgiyanti (2015) reported that the administration of temu ireng (*Curcumae aeruginosae*) extract can increase the body weight of mice. Curcumin in temu ireng (*Curcumae aeruginosae*) corrects problems in the gall bladder by facilitating the release of bile, resulting in increased digestive activity and stimulating the body's metabolism and physiology (Adianti *et al.,* 2020). Therefore, this preparation might also improve body weight problem.

There was no significant difference between absolute organ weight and relative organ weight data in the control group and the dose of 2000 mg/kg which suggest that the polyherbal preparation does not cause changes in the shape of swelling, atrophy and hypertrophy of the organs (Shittu *et al.,* 2015).

We also conducted kidneys histo-pathological scoring since the kidneys important in the xenobiotic elimination. They often involved in the side effects caused by exposure to foreign compounds, including drugs. Estimation of these effects is very important to allow new drugs to be developed (Faria *et al.,* 2019). In preclinical drug development and drug safety assessment, changes in kidney structure and function after drug administration to animals are very important (Radi, 2019). Administration of chloroform extract and temu ireng (*Curcumae aeruginosae*) essential oil for 10 days gave side effects in the form of degeneration and tubular necrosis in the kidney organs of mice (Wiratama *et al.,* 2015). However, our study showed that administration of polyherbal preparation containing garlic (*Aliium sativum*), temu ireng (*Curcumae aeruginosae*) and cardamom (Amomi fructus) did not cause any damage to the kidneys. We suggest that it because the polyherbal preparation used in this study is composed from herbal water extract, a polar solvent. Meanwhile the previous study showing temu ireng (*Curcumae aeruginosae*) toxicity to the kidney was done using chloroform extract, non-polar solven. Therefore, different secondary metabolites are extracted when the solvent used for extraction has different characteristic (Marjawan, 2022).

CONCLUSION

Polyherbal preparations containing garlic (*Aliium sativum*), temu ireng (*Curcumae*

aeruginosae) and cardamom (*Amomi fructus*) shows safety based on an oral acute toxicity test with LD_{50} > 2000 mg/kg.

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