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Research Article

Subchronic Toxicity Test of Kebar Grass Extract of n-Hexane (Biophytum Petersianum Klotzsch) on Weight, ADG and Vital Organ Weight of Rat (Rattus Norvegicus)

Priyo Sambodo[1], Dwi Nurhayati [1], Purwaningsih [1], Trini Susmiati[2], and Claude Mona Airin[3]

[1] Faculty of Animal Husbandry, Papua University
[2] Department of Biochemestry, Faculty of Veterinary Medicine, Gadjah Mada University of Yogyakarta
[3] Department of Physiology, Faculty of Veterinary Medicine, Gadjah Mada University of Yogyakarta

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Email:
monaairin@ugm.ac.id

ABSTRACT

The recent study was carried out to determine the effect of Kebar grass extract of N-hexane (*Biophytum petersianum* Klotzsch) for 28 days peroral on weight, ADG and weight of vital organs of white rat. Forty male and female rats of Sprague Dawley strain aged 8 weeks were examined. The formulated rats were divided into four groups, group I (control), group II, III, IV (treatment), in which each consisted of five rats. The feed were given as much as 15 g/rat/day, while drinking *ad libitum*. In the control group, the rats were given solvent extract, group II, III and IV were given fraction of n-hexane of Kebar grass extract at 1 mg/kg BW, 5 mg/kg BW and 10 mg/kg BW peroral, respectively. Test preparation was given daily for 28 days, observation of toxic symptoms was performed every day while weighing was performed once every two weeks. Based on results obtained in this line of research, the findings indicated that Kebar extract administration for 28 days did not cause toxic symptoms in both male and female rats either in dose II, III or IV. Weight and ADG of male and female rats did not indicate significant differences between the control group and the treatment group.

Keywords: kebar grass, rats, subcronic toxicity test, OECD 407

1. Introduction

The researches on this issue on the potential of *Biophytum* as antihyperlipidemia have received considerable attention and been mostly conducted by various researchers. Renuka *et al.* (2015) stated that ethanol extract, ethanol-water and ethyl acetate of *B. sensitivum* had significant antihyperlipidemic effect in diabetic rats induced by STZ. Kakade *et al.* (2015) added that ethyl acetate extract of *B. sensitivum* (Linn.) had a good activity as hypolipidemic and anti-obesity. The research on the potential of kebar grass as antihyperlipidemia has been performed and indicated a positive result, the fraction of kebar grass extract of n-hexane can decrease the total cholesterol concentration of rabbit blood serum (Sambodo *et al.*, 2015). In the subchronic toxicity test, several parameters to be tested are weight gain, feed intake, drink intake, gross pathology, vital organ weight and histopathology. Weight, ADG and vital organs are the main parameters of preclinical testing; a significant change in these two parameters can be used as an indicator of changes in body physiology. Returning briefly to the above obvious fact, the recent project refers to the OECD 407 subcronic toxicity test with observed parameters of weight and ADG, toxic symptoms and vital organs weight.

2. Material and Method

2.1. Kebar Grass Extraction

Kebar grass powder was immersed in a 50% Ethanol solution and stirred for 30 minutes, then allowed to stand for 24 hours. The next step was filtration to obtain the filtrate. The process was repeated 3 times to obtain the filtrate resulted from 3 times immersion (filtrate I) and residue (Yunita *et al.*, 2009). Next, filtrate I was evaporated with a vacuum rotary evaporator of water bath heater at 70°C, to obtain a concentrated filtrate. The concentrated filtrate was added with n-hexane, then mixed for 5 minutes and allowed to stand for 1 hour then separated by separating funnel. This process was repeated for 5 times to obtain filtrate II and residue. Next, filtrate II was evaporated with a vacuum rotary evaporator of water bath heater at 45°C to obtain Kebar grass extract of n-hexane fraction (Chapagain and Wiesman, 2005; Bogoriani, 2008).

2.2. Oral Subcronic Toxicity Test

Oral subcronic toxicity test was conducted based under OECD 407 (OECD, 2008) and Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia Number 7 of 2014 on the Guidance of Non-Clinical Toxicity Test In Vivo (BPOM, 2014). The experimental animals were male and female Sprague Dawley rats (6-8 weeks old). This research used 40 rats divided into 4 groups, each consisted of 5 experimental animals. The chosen test animals were kept in an individual metabolic cage, feed as much as 15 g per rat per day and drinking ad libitum.

Each group was only given commercial standard feed for 7 days as an adaptation period. The rats were fasted for 15 hours prior to treatment and kept in a metabolic cage for urine collection. After being fasted, the rats were weighed and treated. In group II, III and IV, they were treated with fraction of n-hexane of Kebar grass extract with olive oil solvent of 1 mg/kg BW, 5 mg/kg BW and 10 mg/kg BW, respectively. While group I (control) was not treated. Test preparation was administered daily for 28 days and observation of toxicity symptoms was also performed for 28 days. On day 0, 14 and 29, weighing body weight and weighing the organs were performed on day 29 in all experimental animals. Toxicity observation included: 1). Clinical symptoms observation (mortality calculation, body system observation). 2). Weighing body weight and 3). Weighing internal organs (brain, heart, lung, liver, stomach, intestine, kidney, spleen and testicles).

2.3. Data Analysis

All quantitative data were statistically analyzed. The differences of different treatment effects were analyzed by analysis of variance and significant difference ($P<0.01$), followed by Duncan Multiple Range Test.

3. Result and Discussion

Weight and ADG of female and male rats in group I, II, III and IV on day 0, 14 and 28 were presented in table 1.

Table 1. Average of weigh and ADG rat day 0, 14 th and 20 th after administering kebar extract

	Group	Days to			ADG
		0 (gr)	14 (gr)	28 (gr)	
Female	Control	141,88 ± 8,2	156,94 ± 11,4	152,68 ± 6,8	0,38±0,25
	Dose I	151,58 ± 6,4	164,08 ± 12,6	160,1 ± 13,4	0,3 ± 0,29
	Dose II	144,56 ± 21,8	159,94 ± 26,4	155,56 ± 24,4	0,39 ± 0,2
	Dose III	144,2 ± 17,3	161,58 ± 18,9	156,22 ± 18,9	0,42 ± 0,1
Male	Control	150,72 ± 14,4	189,14 ± 11,1	191,34 ± 13,1	1,31 ± 0,6
	Dose I	158,38 ± 10,5	197,54 ± 10,2	201,3 ± 10,8	1,33 ± 0,3
	Dose II	159,7 ± 7,9	199,28 ± 15,5	199,36 ± 14,7	1,24 ± 0,4
	Dose III	159,66 ± 10,2	199,76 ± 16,3	204,42 ± 18,6	1,44 ± 0,7

In table 1, based on weight of female and male rats, statistical analysis showed no significant differences between control and group I, II and III. This indicated that kebar extract administration for 28 days has no effect on weight gain. The result was in accordance with the previous researches. The result of Oraon and Sinha (2012) research, stated that alcohol extract of *Biophytum reinwardtii* (doses of 50, 75 and 100 mg/kg) did not affect weight of rats. The same result was revealed by Odoh *et al*. (2014), that methanol extract of *Acalypha wilkesiana* containing saponins can fix diabetic rat weight. Anti-obesity mechanism of saponins was by inhibiting lipase enzyme and acting on AMP-activated protein kinase (Marrelli *et al.* 2016).

In this research, toxic symptoms did not appear after administering kebar extract for 28 days. This was in accordance with the result of Oraon and Sinha (2012) research, the acute toxicity test result of alcohol extract of *Biophytum reinwardtii* (doses of 50, 75 and 100 mg/kg) was safe for rats because there were no symptoms of poisoning or death in experimental animals. In the research, average weight organ presented on Figure 2.

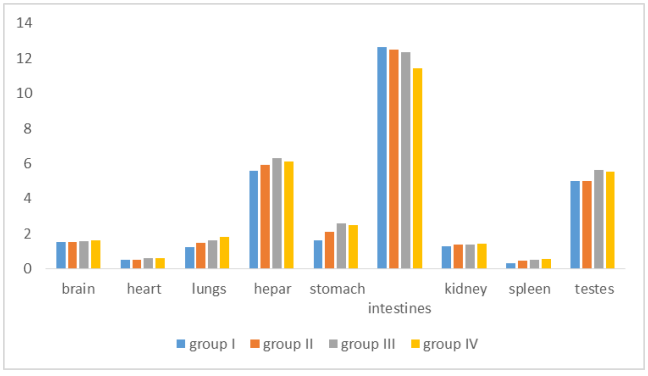

Figure 2. Average weight of experimental animal organs

Based on figure 2, it can be seen that the largest average weight for brain organ was 1.61 g (group IV), heart of 0.60 g (III), lungs of 1.83 g (IV), liver of 6.32 g (III), stomach of 2.59 g (III), intestine of 12.65 g (I), kidney of 1.43 g (IV), spleen of 0.55 g (IV) and testicles of

5.62 g (III). The result of analysis of variance indicated that there was no significant difference between treatment and control groups. This was in accordance with the result of Oraon and Sinha (2012) research, stating that alcohol extract of *Biophytum reinwardtii* (doses of 50, 75 and 100 mg/kg) did not indicate statistically significant differences in both the relative and absolute organs between treatment and control groups. Lathaa *et al.* (2011), mentioned that a dose of 120 mg/kg of saponin-rich *Achyranthes aspera* L. extract caused weight of organs in male Winstar rats to decrease in obese cases.

4. Conclusion

Based on toxicity observation on oral subchronic toxicity test with weight, clinical symptom and internal organ weight parameters, Kebar grass extract of n-Hexana was safe for both male and female Sprague Dawley rats.

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