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## Subchronic Toxicity of the *Physalis minima* Leaves

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### **KEYWORDS**

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**Abstract** The study was to evaluate the subchronic toxicity of aqueous extract of *Physalis minima* leaves in female rats. Eighteen female Wistar rats were orally administered with aqueous extract of *P. minima* leaves at doses of 90, 270 and 450 mg/kg BW, respectively for 90 days. Six female rats without any treatment were provided as a control group. At the end of the experiment, blood samples were collected for hematological and biochemical (aspartate transaminase/AST, alanine transaminase/ALT, creatinine, urea, glucose, cholesterol, and triglyceride) analyses. The results showed that hematological and biochemical biomarkers were not significantly different in treated groups compared to the control group. It is concluded that the aqueous extract of *P. minima* leaves do not induce hematologic toxicity, hepatotoxicity and renal toxicity in the subchronic toxicity study.

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### **Introduction**

*Physalis minima* of the Solanaceae family is some annual herbs and is reported as one of the important medicinal plant in India and other countries (Chothani, et al., 2012). It is commonly called as “ceplukan” in Indonesia and is usually grown as the weed in the crop field. The leaf of *P. minima* is traditionally used as antispasmodic, anthelmintic, antiseptic, analgesic, as well as in the treatment of throat infection (Chothani, et al., 2012; Perk, et al., 2013). It has been reported that *P. minima* contains withanolide, which is a hormone precursor that can be transformed into physiological hormone (Verma & Kumar, 2011).

Previous in vitro study showed that the ethanol extract of *P. minima* leaves at doses of ( $2.5 \times 10^{-3}$  %), ( $5 \times 10^{-3}$  %), and ( $50 \times 10^{-3}$  %) activated the endothelial nitric oxide synthase (eNOS), increased NO release and stimulated cytosolic calcium level (Permatasari, et al., 2010). Besides that, the methanol extract of *P. minima* leaves was reported to possess antibacterial (Chothani, et al., 2012) and anti-inflammatory activities (Kalsum, et al., 2013),

also able to decrease the ventricular fibrosis in ovariectomized rats (Lestari, et al., 2016).

In spite of the extensive use of the herb, there is insufficient scientific evidence validating their efficacy and safety. Most people believe that herbal medicine has no side effects or any potential risks due to the natural origin and is often considered as a substitute treatment and a food supplement (Desai, et al., 2003; Arsad, et al., 2014). However, there are possibilities of toxic effect present due to long-term use and unpredictable amounts of the substance that produces the therapeutic effect (WHO, 2000).

Considering the widespread use of the herb in the management of various diseases, this study was performed to evaluate the subchronic toxicity of the aqueous extract of *P. minima* leaves. Toxicity study should be conducted in accordance with generally accepted principles, as described in the World Health Organization (WHO) Research Guidelines for evaluating the safety and efficacy of herbal medicines (WHO, 2000).

## Material and methods

### *Plant material and extraction*

The leaves of *P. minima* were obtained from Materia Medica, Batu City, Indonesia, then were authenticated at Materia Medica. The fresh *P. minima* leaves were thoroughly washed with distilled water. The cleaned *P. minima* leaves were dried at 40° C in dark condition for 3 days and grounded into fine powder using a miller. One hundred grams (100 g) of *P. minima* dried powder was extracted with 1 liter of boiling water for 1 hour. The extract was filtered with filter paper while the residue was re-extracted under the same conditions twice (Wu, et al., 2006).

### *Animal and treatment*

A total of 24 female Wistar rats weighing approximately 180 to 200 g, obtained from Animal Laboratory of Institut Teknologi Bandung (ITB), Indonesia, were used in this study. The rats were kept in the standard cages of three rats in each cage. All rats were acclimatized at an ambient temperature of 22 ± 2° C with 12 hours light and 12 hours dark cycle for at least seven days prior to the start of the experiment. During the acclimatization and experimental periods, the rats were provided drinking water and normal rat chow ad libitum. The experiment was ethically approved by the Ethics Committee of Faculty of Medicine, Universitas Brawijaya.

The rats were divided into 4 groups (n = 6) for subchronic toxicity study. One control group was without any treatment. Three treatment groups were orally administered with aqueous extract of *P. minima* leaves at doses of 90, 270 and 450 mg/kg BW, respectively for 90 days. During the experiment, the body weight was monitored.

At the end of the experiment, rats were fasted overnight and were euthanized using a lethal dose of ketamine. Blood samples collected from the heart were dispensed into ethylenediaminetetraacetic acid (EDTA) bottles

for hematological analysis using Sysmex Hematology System. Determinations included packed cell volume (PCV), hemoglobin (Hb) concentration, red blood cell (RBC) count, hematocrit (HCT) count, platelet (PLT) count, plateletcrit (PCT) count, white blood cell (WBC) count and differentials, mean capsular volume (MCV), mean capsular hemoglobin (MCH), and mean capsular hemoglobin concentration (MCHC).

A portion of the blood was made some sera used for evaluation of biochemical parameters include aspartate transaminase (AST), alanine transaminase (ALT), creatinine, urea, glucose, total cholesterol, and triglycerides.

### *Statistical analysis*

The results were expressed as the mean ± standard deviation (SD). Data were assessed by two-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc test. Values for which  $p < 0.05$  was considered as statistically significant.

## Results and discussion

In the present study, subchronic toxicity test of *P. minima* leaves aqueous extract on body weight, hematological and biochemical profiles, was performed in female Wistar rats. All rats used for the study appeared normal before, during and post-treatment. Mortality was not recorded at all dose level.

The effect of *P. minima* leaves aqueous extract on the body weight of treated rats compared with control (without treatment) is shown in Table 1. There were no significant differences ( $p > 0.05$ ) in increasing body weight among the four groups. The changes in body weight serve as a sensitive indicator of the general health status of animals and usually reflect physiological changes (Arthur, et al., 2011; Perk, et al., 2013). In this study, the increasing body weight is in accordance with the growth and development of animals. It can be stated that *P. minima* leaves aqueous extract did not

interfere with the normal metabolism of animals as corroborated by non-significant differences ( $p > 0.05$ ) from animals in the control group.

The effect of *P. minima* leaves aqueous extract on hematological profile in female Wistar rats is shown in Table 2. All hematologic parameters showed no significant differences ( $p > 0.05$ ) between control and treated rats. The changes in hematological parameters found in the animal study provide a prediction of toxicity on the hematological system in humans (Olson, et al., 2000). The lack of significant changes in hematological parameters in this study indicates the safety of *P. minima leaves aqueous extract*. The normal value of hemoglobin in female Wistar rats could be used to justify the fact that *P. minima leaves aqueous extract* at all dose does

not induce anemia, making it is safe to be used (Arthur, et al., 2011).

The effect of *P. minima* leaves aqueous extract on biochemical parameters of treated rats compared with control is shown in Figure 2, 3, and 4. There were no significant differences ( $p > 0.05$ ) in serum AST and ALT levels between control and treated rats. The levels of AST and ALT were measured to assess the hepatotoxicity. Increased AST and ALT levels are associated with hepatic damage or the changes in the permeability of the hepatocyte membrane. The ALT is considered a more specific enzyme for liver function (Perk, et al., 2013). The results suggest that *P. minima leaves aqueous extract* does not induce hepatotoxicity.

**Table 1.** The effect of *P. minima* leaves aqueous extract on body weight in control and treated rats during subchronic toxicity study (mean  $\pm$  SD (n=6))

Duration of Treatment	Control	<i>P. minima</i> leaves aqueous extract		
		90 mg/kg BW	270 mg/kg BW	450 mg/kg BW
Month 0	192.4 $\pm$ 16.76 g	201.0 $\pm$ 19.07 g	201.9 $\pm$ 13.88 g	192.2 $\pm$ 12.56 g
Month 1	202.9 $\pm$ 17.74 g	211.5 $\pm$ 19.34 g	212.4 $\pm$ 13.88 g	202.7 $\pm$ 12.55 g
Month 2	216.7 $\pm$ 18.95 g	225.8 $\pm$ 22.30 g	228.6 $\pm$ 17.78 g	208.5 $\pm$ 11.33 g
Month 3	220.8 $\pm$ 18.64 g	229.4 $\pm$ 21.49 g	232.9 $\pm$ 19.82 g	214.2 $\pm$ 12.66 g

**Table 2.** The effect of *P. minima* leaves aqueous extract on hematological profiles in control and treated rats during subchronic toxicity study (mean  $\pm$  SD (n=6))

Parameters	Control	<i>P. minima</i> leaves aqueous extract		
		90 mg/kg BW	270 mg/kg BW	450 mg/kg BW
WBC ( $10^3/\mu\text{L}$ )	5.77 $\pm$ 1.46	4.88 $\pm$ 1	4.95 $\pm$ 0.84	4.30 $\pm$ 0.72
RBC ( $10^6/\mu\text{L}$ )	5.98 $\pm$ 0.4	5.68 $\pm$ 0.48	5.53 $\pm$ 0.48	5.80 $\pm$ 0.59
Hemoglobin (g/dL)	13.62 $\pm$ 0.51	12.98 $\pm$ 0.66	12.90 $\pm$ 0.26	12.97 $\pm$ 0.61
Hematocrit (%)	34.83 $\pm$ 2.38	34.13 $\pm$ 2.23	33.57 $\pm$ 2.04	35.00 $\pm$ 1.15
PLT ( $10^3/\mu\text{L}$ )	575.83 $\pm$ 112.98	543.67 $\pm$ 152.24	588.67 $\pm$ 81.84	593.00 $\pm$ 95.41
PCT (%)	0.323 $\pm$ 0.062	0.303 $\pm$ 0.07	0.346 $\pm$ 0.044	0.347 $\pm$ 0.057
MCV ( $\mu\text{m}^3$ )	56.67 $\pm$ 3.88	58.83 $\pm$ 2.48	59.67 $\pm$ 1.37	57.33 $\pm$ 1.63

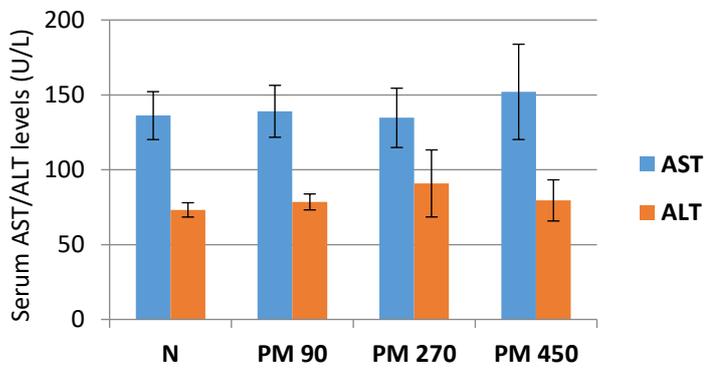
WBC: white blood cell count, RBC: red blood cell count, PLT: platelet count, PCT: plateletcrit count, MCV: mean capsular volume.

There were no significant differences ( $p > 0.05$ ) in the levels of serum urea and creatinine between control and treated rats. Serum urea and creatinine levels were measured to assess the renal toxicity. The results suggest that *P. minima leaves aqueous extract* does not induce renal toxicity.

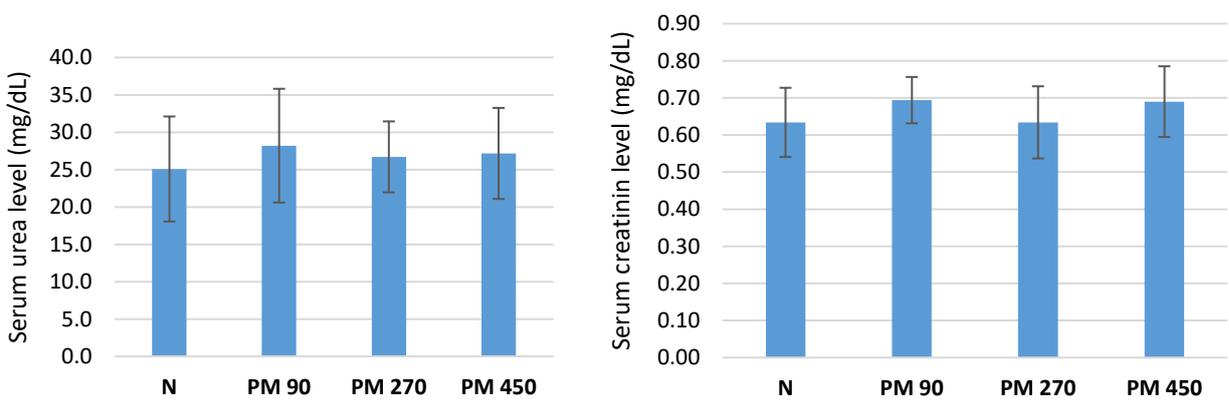
The effect of *P. minima leaves aqueous extract* on lipid metabolism was assessed by measuring total cholesterol and triglycerides levels. There were no significant differences ( $p > 0.05$ ) in total cholesterol and triglycerides levels between control and treated rats. The

results indicate that *P. minima leaves aqueous extract* had no effect on lipid metabolism.

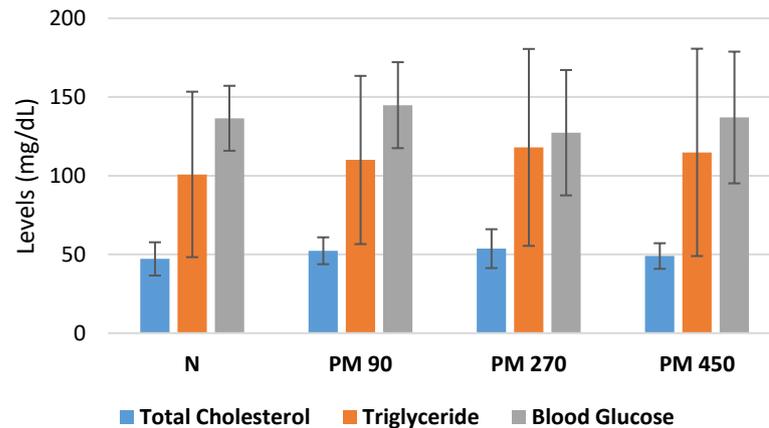
In addition, carbohydrate metabolism was assessed by measuring the blood glucose level. There was no significant difference ( $p > 0.05$ ) in blood glucose level between control and treated rats. The result indicates that *P. minima leaves aqueous extract* does not induce hyperglycemia nor hypoglycemia. This is contrary to the other study that reported the ethanol extract of *P. minima* had hypoglycemic effect by oral administration (Chotani, et al., 2012).



**Figure 2.** The effect of *P. minima leaves aqueous extract* on serum AST and ALT levels in subchronic toxicity study. It shows that the extract administration for 90 days does not induce hepatotoxicity. N: normal; PM 90, PM 270, PM 450: *P. minima leaves aqueous extract* at doses of 90, 270, and 450 mg/kg BW.



**Figure 3.** The effect of *P. minima leaves aqueous extract* on serum urea and creatinin levels in subchronic toxicity study. It shows that the extract administration for 90 days does not induce renal toxicity. N: normal; PM 90, PM 270, PM 450: *P. minima leaves aqueous extract* at doses of 90, 270, and 450 mg/kg BW.



**Figure 4.** The effects of *P. minima* leaves aqueous extract on total cholesterol, triglyceride, and blood glucose levels in subchronic toxicity study. It shows that the extract administration for 90 days does not affect lipid and glucose metabolisms. N: normal; PM 90, PM 270, PM 450: *P. minima* leaves aqueous extract at doses of 90, 270, and 450 mg/kg BW.

## Conclusion

In general, this article can show new results on toxicology test of subchronic dosage of *P. minima* leaves aqueous extract. Results of the present study suggest that *P. minima* leaves aqueous extract may not change body weight and may not lead hematological, hepatic and renal toxicities, also may not affect lipid and glucose metabolism in female Wistar rats.

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