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## Research Article

### PRELIMINARY ACUTE ORAL TOXICITY STUDY OF THE WATER EXTRACT OF *PHYSALIS MINIMA* LEAVES

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#### ABSTRACT

*Physalis minima* has been used in traditional medicine for the treatment of different ailments. Thus, it is necessary to evaluate the toxicity of this plant's extract to prevent any unwanted side effects. This study aimed to determine the acute oral toxicity level of water extract of *P. minima* leaves on female Sprague Dawley rats at a dosage of 2000 mg/kg for a period of 14 days using the Organisation for Economic Co-Operation and Development (OECD) Guideline 423. The findings showed no mortality and no change in behavioural pattern. Besides, the body weight of rats increased throughout the study. The rats' internal organs showed a normal histopathological architecture compared to the control group. Hence, it can be concluded that the oral administration of water extract of *P. minima* did not produce any significant toxic effects on rats.

**Keywords:** Acute oral toxicity; Water extract; Female Sprague Dawley Rats; Histopathology; *Physalis minima*

#### INTRODUCTION

Nowadays, herbal medicines are widely accepted as an alternative medicine in healthcare due to its natural origin. Therefore, through toxicity studies, the toxicology of plant extracts can be determined to safeguard people's health and raise public awareness on toxicity. Toxicology study is significant in the pre-clinical safety evaluation of medicines before it can be implemented and assessed in humans. It also helps to determine whether a new drug should be adopted for clinical use or not<sup>1</sup>. According to Chinedu et al, acute toxicity is defined as the unwanted effect that occurs either immediately or at a short time interval after a single or multiple administration of a substance within 24 hours<sup>2</sup>. Usually, the effects will be observed on day 7 or 14 after dosing, but occasionally it can be detected as early as 24 hours<sup>3</sup>.

Plants contribute many essential nutrients, vitamins and chemical compounds. Therefore, plants play an important role in the development of new drugs for the treatment and prevention of diseases<sup>4-7</sup>. *Physalis minima* is a member of the Solanaceae family and is known as letup-letup in Malaysia. Besides Malaysia, it is also widely found in Singapore, Australia, India, Baluchistan, Afghanistan and Tropical Africa<sup>8</sup>. This plant has been used in various local remedies as a diuretic and antipyretic compound<sup>9</sup> and to treat ailments like headache, fever, hypertension, diabetes and lower abdominal pain<sup>10</sup>. Moreover, it has also shown anti-cancer activities<sup>11</sup>.

To date, no study has been done to investigate the toxicity of *P. minima* leaves water extract. Many people believe that since herbal medicine originates from nature it does not possess any side effects or toxic effects. Therefore, toxicity analysis should be carried out to document herbal plants to ensure that consumers can benefit from herbal medicine without neglecting possible toxic effects. The toxicology evaluation of *P. minima* leaves water extract is very important to recognise the side effects, especially for people who consume it as a supplement or

medicine. This study is in line with Stanković et al. (2016) who reported that traditional medicine in the form of water extract is used more often compared to alcoholic extract in oral applications<sup>12</sup>. Hence, the purpose of this study is to investigate the possible acute toxic effects of *P. minima* water extract in female rats according to the Organization for Economic Co-Operation and Development Guideline (OECD) Guideline 423<sup>13</sup>.

#### MATERIALS AND METHODS

##### Plant material

*P. minima* leaves were obtained from Kepala Batas, Penang, Malaysia. Voucher specimens of this plant were deposited in the Herbarium Unit, School of Biological Sciences, Universiti Sains Malaysia (voucher number 11723).

##### Preparation of extract

The leaves were first washed with tap water to remove dirt and then thoroughly rinsed with distilled water. Next, the leaves were cut into small pieces and soaked in distilled water at a 1:10 ratio for 24 hours. The obtained extract was agitated using a shaker at room temperature. After that, it was filtered and transferred to a round bottom flask, labelled and immediately subjected to freeze-drying for four days. Finally, the extract was stored at -20°C until further use.

##### Experimental design for acute oral toxicity

##### Animal

The study was performed after getting approval from Animal Ethics Committee, Universiti Sains Malaysia (AECUSM). Ref. No: USM/Animal Ethics Approval/2014/593. Six healthy young nulliparous and non-pregnant female rats (*Rattus norvegicus*) of the Sprague Dawley strain were obtained from the animal house facility of Animal Research Centre of Advanced Medical and

Dental Institute, Universiti Sains Malaysia, Penang, Malaysia. Female rats were selected for this study because we would like to further our study by using *P. minima* extract to inhibit the proliferation of breast cancer cells in female rats. To sustain and control the test facility's environment, the rats were housed one per cage under controlled temperature ( $24\pm 1^\circ\text{C}$ ), relative humidity (30% to 40%) and lighting (12/12-hour light-dark cycle) with free access to water and food. The OECD Guideline 423 for laboratory animals were followed. Experimental cages were tagged with a label corresponding to the rat in it, while permanent ink of different colours was used to identify individual rats by marking their tails. The rats were acclimatised in the experimental room for five days and their feeding and drinking were checked before commencing the study.

#### Dose administration

*P. minima* water extract (2000 mg/kg) was administered using the oral gavage technique. According to OECD (2001), a limit dose of 2000 mg/kg is essential for the member countries for imposition. However, there are ranges of 2000 to 5000 mg/kg for substance in few countries which have a requirement for information on toxicity at dose levels<sup>25</sup>. This research followed Tammu et al. (2013) for the limit test of 2000 mg/kg since the methanolic extract of *P. minima* leaves was found to be non-toxic as it did not cause any toxic symptoms and mortality up to the dose of 2000 mg/kg<sup>15</sup>.

#### Acute oral toxicity test

The rats were subjected to a fasting period of 12 hours prior to the oral administration of a single dose of *P. minima* water extract. Three rats were administered with a single dose of 2000 mg/kg via oral gavage. The rats were continuously monitored for 12 hours after dosing for any abnormalities in general behaviour and signs of toxicity or death. After dosing, each rat was observed for clinical signs of abnormal behavioural. The body weight, food consumption, fluid intake and behavioural pattern of rats were recorded each day until day 14.

#### Histopathology

At day 15, the rats were humanely euthanized by using carbon dioxide<sup>24</sup>. Vital organs such as kidney, liver, brain and lung were carefully excised and any adherent tissues were trimmed off. The excised organs were fixed in 1.23 mol/L of 10% neutral buffered formalin (pH 7.4). The tissues were processed by first clearing it in xylene, followed with graded alcohol and then embedded in paraffin, sectioned into 3  $\mu\text{m}$  slices and stained with haematoxylin and eosin. Slides were cover-slipped with a thin glass and an appropriate adhesive mounting medium (DPX) was used for overall morphological evaluation. Staining preparation was performed whereby slides were deparaffinized in xylene and rehydrated through a serial dilution of alcohol (95%, 80%, 70% and 50%) and water. Slides were then immersed in Harris haematoxylin for 20 min before rinsing with water. After rinsing, the slides were differentiated in 0.5% acid alcohol for 2 to 3 dips before rinsing with water for 2 min. Next, slides were placed in 0.3% ammonia until the tissue section turned bright blue in colour. Then, the slides were thoroughly washed and dipped in 95% alcohol. Finally, the slides were counterstained with eosin and then dehydrated in 95% alcohol for 30 dips, followed by dehydrating it through a serial solution of absolute alcohol, clearing with xylene and drying and mounting in DPX.

## RESULTS

#### Body weight, food consumption, fluid intake and behavioural pattern of rats

The body weight of rats in both control and treated groups progressively increased throughout the study period, as shown in Table 1. Besides that, all rats appeared consistently healthy throughout the 14 days of experiment. During treatment, the rats continued with their regular food and fluid intake, which caused weight gain over the course of the study. No death was recorded and no other significant observation deviant from the norm was seen in the rats. On the other hand, during behavioural observation of the rats after dosing, an elevated respiration rate for the first 30 min was noted in the extract treated group. In addition, an increase in somatomotor activity was observed for the first 30 mins and at 4 h in this treated group. This could have occurred due to the stress of receiving the oral administration of the extract. The behavioural observations are summarised in Table 2.

#### Histopathology analysis

Rats were subjected to a detailed post-mortem examination of internal organs, which did not reveal any macroscopic differences in size, colour or texture. A single dose administration of the 2000 mg/kg of *P. minima* extract did not affect the brain, kidney, liver and lungs as it did not cause lethality throughout the 14 days of treatment. Fig. 1 illustrates the histopathological examination of the treated rats' brain, kidney, liver and lungs stained with haematoxylin and eosin stain, which showed no remarkable lesions that could be attributed as the effect of a single dose of *P. minima* leaves water extract. Fig. 1(a) and (b) show normal Purkinje cells without any degeneration. Besides, both grey and white matter were revealed to be normal under microscopic examination. Likewise, no empty cytoplasm, no damage to neurons and no nuclear shrinkage were observed. Fig. 1(c) represents the kidney section, which shows normal cells lining the Bowman's capsule. Next, in Fig. 1(d), the liver section containing the portal tract, portal vein, portal artery, bile duct, sinusoids and hepatocytes are shown. No abnormal morphologies were seen in all these cells compared to the control group. Fig. 1(e) shows the lung section, whereby alveolar spaces, alveolar wall and intact bronchiolar epithelial cells with normal morphology were observed. Clara cells or non-ciliated bronchiolar cells located in the bronchiolar epithelium presented no undifferentiated epithelial cells.

## DISCUSSION

Acute oral toxicity test performed on rats can be used to evaluate natural remedies' various pharmacological activities. This test is widely used to identify and classify the substances that can cause acute damage to living organisms in high doses<sup>14</sup>. Thus, it is very important to ensure that a definitive assessment of the safety of medicinal plants is conducted when the medicinal plants have enough potential for development into pharmacological products<sup>14</sup>.

There is no published report on the toxicity of *P. minima* water extract despite the extensive use of this plant in various herbal remedies. However, Tammu et al. (2013) have performed an anti-ulcer activity analysis of *P. minima* using methanol extract<sup>15</sup>. In this research, no rats exhibited any signs of clinical toxicity due to a single oral dose of 2000 mg/kg of *P. minima* water extract and the treatment did not alter their feeding or fluid intake. This indicates that *P. minima* extract can be categorised as a non-toxic material with no clinical toxicity when orally administered for a

short time<sup>16</sup>. In clinical settings, a weight loss of more than 10% of the initial body weight is considered scientifically significant<sup>17, 18</sup>. Rats treated with *P. minima* water extract continued to gain weight throughout the 14 days of experiment. According to Angelina et al. (2012), the alteration of body weight gain and organ weights of the treatment groups compared to the control group will reflect the toxicity of the substance<sup>19</sup>. In this study, there were no significance differences in body weight gain with control group in the result analysis in  $p \geq 0.05$ . This concurs with the acute oral toxicity evaluation study by Saleem et al. (2017) and Ardiana et al. (2017), who reported that animals administered with *Saccharum munja* and *Camellia sinensis* extract respectively, had normal body weight gain and food and fluid intake for 14 days<sup>20, 21</sup>. In this study, no lesions or changes in colour for both the control and treated groups were found during macroscopic observation of organs.

Moreover, no abnormalities were discovered in the tissues during the histological examination of treated rats compared to the control rats. This finding agrees with the report by Bigliani et al. (2010) who observed no abnormalities during histological examination of rats treated with water extract from *Larrea divaricate*<sup>22</sup>. Apart from that, Kamonwannasit et al. (2013) reported no signs of toxicity in water extract of *Aquilaria crassna* at the dose of 2000 mg/kg<sup>23</sup>. Since the present study is the first experiment conducted to examine the acute oral toxicity of *P. minima* water extract in rats, the findings are crucial. Nevertheless, it is too early to confirm the safety of *P. minima* because it has been only tested in an experimental animal. However, this investigation has contributed some information on toxicity that can be applied in future research.

**Table 1: Effects of the extract on body weight, food consumption and fluid intake of rats in acute toxicity study**

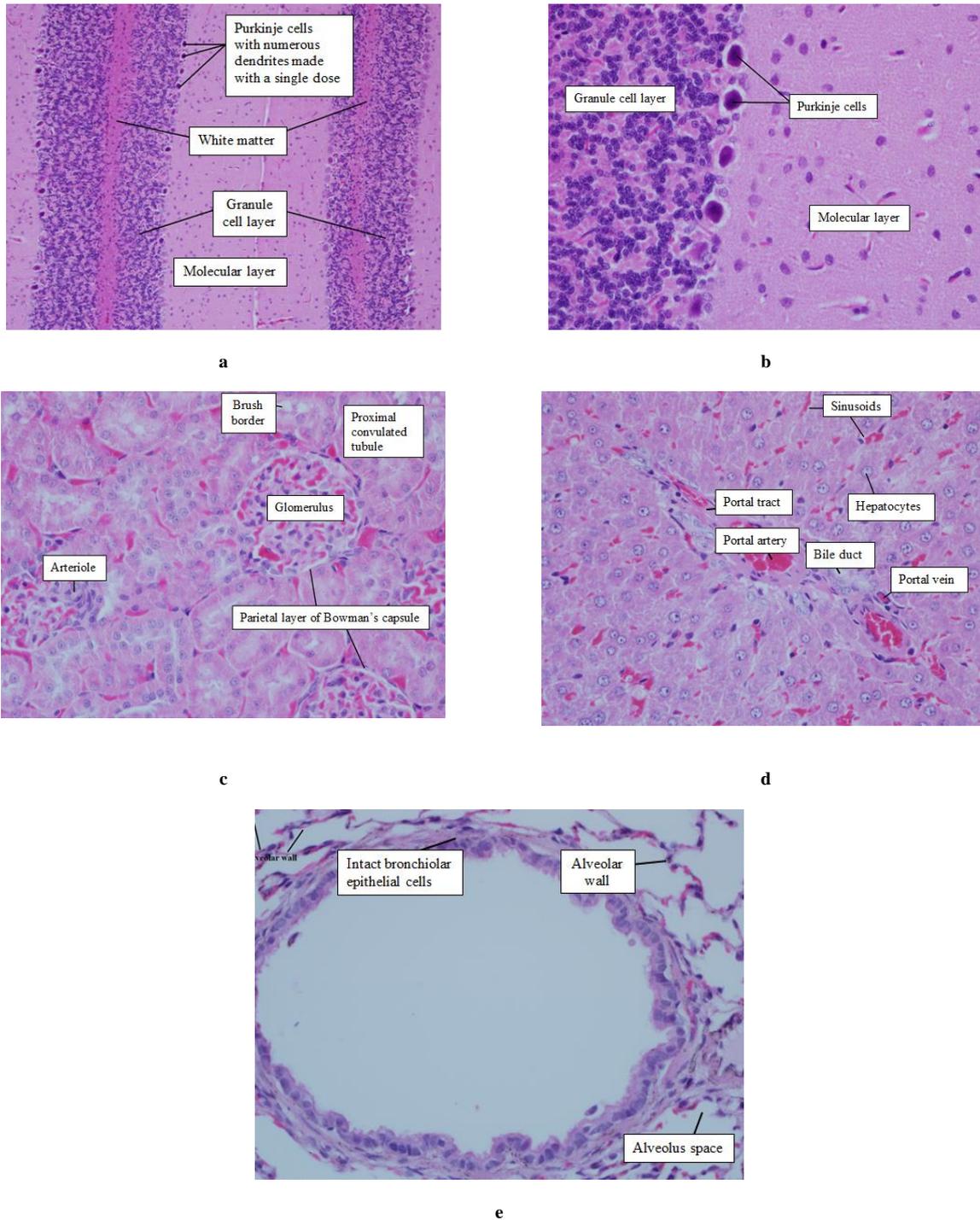
Groups	Control	2000 mg/kg PMWE
<b>Body weight (g)</b>		
1 <sup>st</sup> Day	256.67±15.27	229.00±10.47
7 <sup>th</sup> Day	257.72±6.70	230.85±4.43
14 <sup>th</sup> day	260.55±11.56	233.20±7.03
<b>Food consumption (g)</b>		
1 <sup>st</sup> Day	16.90±4.11	15.38±3.72
7 <sup>th</sup> Day	17.62±1.15	16.10±1.59
14 <sup>th</sup> day	17.66±2.64	16.71±2.33
<b>Fluid intake (mL)</b>		
1 <sup>st</sup> Day	25.74±5.58	25.81±5.41
7 <sup>th</sup> Day	21.85±2.08	27.10±3.46
14 <sup>th</sup> day	26.62±2.86	27.15±1.79

Note: PMWE: *P. minima* water extract; Values are presented as mean ± SEM; N=3.

**Table 2. Behavioural patterns of rats in extract treated at dose 2000 mg/kg and control groups**

Parameters	Observations of control and <i>P. minima</i> leaves extracts treated groups											
	30 mins		4h		24h		48 h		7 days		14 days	
	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG
Fur and skin	N	N	N	N	N	N	N	N	N	N	N	N
Eyes and Nose	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Respiration	N	I	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N
Faeces consistency	N	N	N	N	N	N	N	N	N	N	N	N
Somatomotor activity and behaviour pattern	N	I	N	I	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N
Convulsions and tremors	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Coma	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Mortality	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

Note: CG: Control groups; TG: *P. minima* extract groups; N: Normal, P: Present, I: Increased, NF: Not found



**Fig. 1:** Photomicrograph image of the (a) and (b) brain, (c) kidney, (d) liver and (e) lung of water extract of *P. minima* treated groups at dose 2000 mg/kg stained with haematoxylin and eosin. (a) Scale bar 100  $\mu\text{m}$  (x10) while (b) to (e) scale bar 20  $\mu\text{m}$  (x40)

## CONCLUSION

This study showed that *P. minima* water extract is non-toxic via the oral acute toxicity test performed on rats. Nonetheless, further studies should be conducted to perform biochemical and haematological analyses of rats treated with water extract of *P. minima*.

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