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ACUTE ORAL TOXICITY STUDY OF ROOT METHANOL EXTRACT OF *Goniothalamus lanceolatus* Miq. AND ITS ISOLATED BIOACTIVE COMPOUND (PARVISTONE D) IN MURINE MODEL

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ABSTRACT

Goniothalamus lanceolatus Miq. is widely used by the indigenous people of Sarawak, Malaysia as a folk remedy to treat various ailments including skin diseases, cold, and fever. A previous study reported that the root methanol extract, and parvistone D, an active compound of the plant, showed promising *in vitro* antiplasmodial activity against *Plasmodium* parasites. However, there is limited data reporting on its toxicological profile. Thus, this study aims to evaluate the potential toxicity of root methanol extract and parvistone D of *G. lanceolatus* in mice. The acute oral toxicity of the extract and compound was assessed at a single dose of 2000 and 500 mg/kg body weight, respectively. The animals were observed for any mortality, behavioral, motor-neuronal abnormalities, and body weight changes for 14 days. At the end of the experiment, relative organ weights were measured, and gross examination, as well as histopathological analysis, were performed. There was no sign of toxicity, and mortality seen in mice treated with *G. lanceolatus* root methanol extract, and parvistone D at the administered doses. In addition, no significant differences were observed in the body and relative organ weights between the control and treated groups. Gross and histopathological examinations showed normal appearance of the liver, spleen, kidneys, heart, and lungs as compared to their respective control groups. In conclusion, oral administration of root methanol extract, and parvistone D of *G. lanceolatus* are safe at the studied dosage levels and cause no acute toxicity in mice.

Key words: Acute oral toxicity, *Goniothalamus lanceolatus*, mortality, parvistone D, root methanol extract

INTRODUCTION

Medicinal plants have been widely embraced as an alternative to allopathic medicines in many countries for the treatment of various diseases (Nfozon *et al*., 2019; Gouws *et al*., 2020). Functional bioactive compounds of plant origin have been the source of innumerable therapeutic agents in drug discovery (Yuan *et al*., 2016; Mustafa *et al*., 2017; Dawurung *et al*., 2021). However, some medicinal plants are inherently toxic by their active constituents and can cause adverse reactions if inappropriately used (WHO, 2019). Therefore, it is of utmost importance to assess the toxicity of medicinal plants to facilitate a better understanding of the risks associated with the use of the plants, and to ensure safety.

Goniothalamus lanceolatus Miq. is native to Borneo, especially the rainforest jungle of Sarawak, Malaysia. It is a member of the Annonaceae family and has been used by the indigenous people of Sarawak to treat colds, fever, and skin diseases. Different parts of the plants are used for various ailments. A decoction of the leaves and stems is taken orally to help reduce fever and relieve symptoms of colds. In addition, the roots are boiled and used as an aromatic steam bath to treat skin diseases (Wiart, 2007). The plant crude

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extracts were reported to possess *in vivo* antimalarial properties and *in vitro* antiproliferative activities against several tumor cell lines (Zohdi *et al*., 2017; Razali *et al*., 2021). Phytochemical investigations of the plant afforded several new and known styryl lactones which were reported to have cytotoxic effects against selected human lung and colorectal cancer cells (Rasol *et al*., 2018; Bihud *et al*., 2019).

A recent study reported that the root methanol extract of *G. lanceolatus* and the isolated styryl lactone, namely parvistone D were shown to possess *in vitro* antiplasmodial activity against *Plasmodium* parasites (Kaharudin *et al*., 2020). The study suggested that the extract and compound are potential candidates to be further exploited as a source of antiplasmodial agents. Furthermore, a preliminary study done by Zohdi *et al*. (2017) reported that the extract did not produce any observable symptoms of toxicity and mortality at the dose of 300 mg/kg. Despite these interesting findings, the related toxicological information such as body weight change, organ weights, and histopathological analysis are limited. A toxicity study using a selected animal model is pivotal to predict the adverse effect and determine the safety level of a tested substance (Katzung *et al*., 2012; Olayode *et al*., 2020). Thus, this study was carried out to evaluate the acute oral toxicity profile of *G. lanceolatus* root methanol extract and its isolated bioactive compound parvistone D in mice. Results from acute toxicity studies generally serve as a guide in dosage selection for the subsequent *in vivo* antimalarial study (Akhila *et al*., 2007).

MATERIALS AND METHODS

Plant collection, extraction, and isolation

The roots of *G. lanceolatus* were collected from Sarawak, Malaysia, in June 2012. The plant sample was identified and authenticated by the late Professor Dr. Kamaruddin Mat Salleh. Voucher specimen FBAUMS 108 was deposited at the herbarium of Universiti Malaysia Sarawak for reference purposes. The root methanol extract and parvistone D of *G. lanceolatus* were prepared following the protocol described earlier (Kaharudin *et al*., 2020). Briefly, 4 kg of dried, ground roots of *G. lanceolatus* were successively extracted by maceration with methanol for 48 hours at room temperature. The extract was evaporated to dryness using a rotary evaporator at 50 – 70 °C to give the methanol crude extract of the roots. Parvistone D was isolated and purified using preparative high-performance liquid chromatography (HPLC) and recycling HPLC as described by Rasol *et al*. (2018).

Experimental animals

Five-week-old ICR mice of both sexes, weighing 25-30 g, were obtained from Laboratory Animal Facility and Management (LAFAM), Universiti Teknologi MARA (UiTM) Selangor Branch, Puncak Alam Campus. The animals were allowed to acclimatize for one week in the animal house at 25 ± 2 °C on a 12 h light-12 h dark cycle. Male and female mice were housed separately with three mice per polycarbonate cage. The animals had free access to a standard pellet diet and water *ad libitum*.

Acute oral toxicity study

This study was performed according to the Organization for Economic Cooperation and Development (OECD) 423 guidelines (2001) (OECD, 2001). Since the previous study reported no mortality at the dose of 300 mg/kg, thus the starting dose for the current study was selected to be 2000 mg/kg body weight for root methanol extract and 500 mg/kg body weight for parvistone D. A total of 18 mice were randomly divided into 3 groups, with 6 mice (3 males & 3 females) per group. The mice fasted overnight before treatment but the water was not withheld. The *G. lanceolatus* root methanol extract and parvistone D were administered by oral gavage as a single dose of 2000 mg/kg, and 500 mg/kg body weight (bwt), respectively. The control group received 5% Tween 60, which was used to suspend the extract and compound. The administered volume for all treatments was adjusted to 0.2 mL/kg bwt for each mouse.

Cage-side observations and body weight

Each mouse was continuously monitored for the first hour after dosing, followed by intermittent observation every 2 h for a period of 24 h, and then daily for 14 days. The parameters of interest were changes in skin and fur, eyes and mucous membranes, respiratory pattern, the autonomic nervous system features such as salivation, diarrhea, and urination, central nervous system features such as tremors, convulsions, and ptosis. Changes in gait and posture were also monitored along with stereotypical activities such as excessive grooming and repetitive circling. The body weight of each mouse was measured on the first day of treatment, and weekly thereafter. All observations were systematically recorded and maintained with an individual record.

Gross examination and relative organ weight

After 14 days of the observational period, the mice were euthanized by cervical dislocation. Selected vital organs such as liver, spleen, kidneys, heart, and lungs were collected, washed immediately with normal saline, and dried using blotting paper. The gross examination of these organs was carried out to detect the possible development of lesions or other abnormal signs. The relative organ weight of each animal was calculated using Equation 1:

$$\text{Relative organ weight (\%)} = \left(\frac{\text{organ weight (g)}}{\text{body weight of mice on the day of sacrifice(g)}}\right) \times 100$$

Histopathological analysis

The liver, spleen, kidneys, heart, and lungs were fixed in 10% buffered formalin and subsequently embedded in paraffin wax. Sections of 3 - 5 µm thickness were prepared using a rotary microtome (Model RM2235, Leica, Germany) and stained with hematoxylin and eosin (H&E) using an autostainer (Model ST5010, Leica, Germany). The histopathological analysis was performed in a blinded manner at 200x magnification using a Leica DM2000 light microscope equipped with an ICC50 HD camera (Leica, Germany).

Statistical analysis

The data were analyzed using GraphPad Prism version 6.0 (GraphPad Software Inc., San Diego, CA, USA). A repeated-measures analysis of variance (ANOVA) was performed to determine the effects of treatment and time. The values were expressed as mean ± standard deviation (SD). A p-value<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The present study reported on the acute toxicity of root methanol extract and parvistone D of *G. lanceolatus* which were previously reported to have promising *in vitro* antiplasmodial activity. In this study, acute toxicity testing is essential to evaluate the approximate safe dosage of the extract, and compound so that it can be used as a reference for future research. The acute toxicity study evaluates the short-term adverse effects of tested substances when administered in a single dose during a period of 24 h (Erhirhie *et al*., 2018). It is usually administered via oral routes to determine the median lethal dose (LD_{50}) for a particularly toxic substance performed principally in rodents (Kaid *et al*., 2019). There was no mortality recorded during the acute oral toxicity study of root methanol extract, and parvistone D of *G. lanceolatus* at 2000 and 500 mg/kg bwt, respectively (Table 1). The skin, fur, eyes, behavioral pattern such as gait, and posture as well as autonomic and central nervous system activities of mice treated with the extract, and compound remained unchanged when compared to the control group. Hence, the approximate lethal dose (LD_{50}) of *G. lanceolatus* root methanol extract, and parvistone D was estimated to be higher than 2000 and 500 mg/kg bwt, respectively.

Changes in body weight are a basic indicator to assess the general health and metabolic status of animals (Jothy *et al*., 2011). It has been used as a reference to indicate early signs of toxicity caused by various chemicals, and drugs (Ekanayake *et al*., 2019). In the present study, there was a gradual increase in the percentage of body weight of treated mice which was comparable to the control group during the study period (Figure 1). However, there was no significant difference (p>0.05) in the body weights of treated mice, and the control group of both sexes. The results indicate that the extract and compound did not exert any deleterious effects on the general health status, and metabolic growth of the mice.

Table 1. Effects of root methanol extract and parvistone D of *G. lanceolatus* on mortality and clinical changes in mice during 14-day oral acute toxicity study

Treatment Groups	Mortality	Clinical changes
Male		
Control	0/3	Nil
Root methanol extract	0/3	Nil
Parvistone D	0/3	Nil
Female		
Control	0/3	Nil
Root methanol extract	0/3	Nil
Parvistone D	0/3	Nil

The gross examinations were performed to determine the presence of any visible lesion on vital organs such as the liver, spleen, kidneys, heart, and lungs, which are mainly affected by metabolic reactions caused by toxicants (Dybing *et al*., 2002). The color, texture, and size of these internal organs are some of the initial indications of organ toxicity, induced by toxic compounds (Ekanayake *et al*., 2019). In this study, no lesions, or changes in color, shape, size, and texture were observed in all vital organs of treated mice as compared to the control group of both sexes (Figures 2 & 3). The results indicate that the extract and compound did not cause any adverse effects on all primary organs of tested mice.

The relative organ weight index is used as a basic indicator to assess the adverse effects of tested drugs or chemicals in particular organs (Pariyani *et al*., 2015). Organ weight is one of the most sensitive drug toxicity indicators, and its changes often precede morphological changes (Piao *et al*., 2013). In the present study, there were no significant (p>0.05) changes observed in the relative organ weights of treated mice of both sexes compared to their respective control groups (Table 2). The results showed that the extract and compound did not produce any remarkable toxic effect on the relative organ weights. These observations were further confirmed by the histopathological assessment of the organs shown in Figures 4 and 5.

The histopathological evaluation of the liver, spleen, kidneys, heart, and lung tissue sections of treated mice of both sexes did not show any lesions or abnormal histopathological changes as compared to their respective control groups (Figures 4 & 5). The liver sections in treated mice showed normal radiating hepatocytes interspaced with hepatic sinusoids and normal appearance of Kupffer cells. As for the spleen, all treated mice showed intact spleen follicles

with distinct white and red pulps. The kidney tissue section of treated mice exhibited normal architecture similar to the control group with intact Bowman's capsule with no sign of interstitial and intraglomerular congestion. Similarly, no abnormalities were observed in the heart, and lungs of the treated mice as compared with those in the control group. The heart showed normal cardiac muscle fibers and the lungs showed normal alveolar structure in both the control and treated mice. The histopathological examinations are in good supportive correlation with the findings from relative organ weight, and gross examinations. It showed that root methanol extract and parvistone D did not cause any remarkable alteration or damage to the vital organs.

Table 2. Effects of root methanol extract and parvistone D on the relative organ weight in mice during a 14-day oral acute toxicity study

Treatment Groups	Relative organ weight (%)				
	Liver	Spleen	Kidneys	Heart	Lungs
Male					
Control	5.56±1.01	0.49±1.02	1.00±0.90	0.58±1.32	0.97±1.25
Root methanol extract	5.73±1.15	0.40±0.91	1.06±1.12	0.54±0.92	1.08±1.02
Parvistone D	5.85±1.06	0.41±1.02	1.03±1.11	0.57±1.10	0.90±1.30
Female					
Control	5.42±1.10	0.43±0.92	0.76±1.18	0.50±1.13	0.94±1.14
Root methanol extract	5.48±1.28	0.41±1.22	0.71±1.25	0.56±1.40	1.05±1.51
Parvistone D	5.34±1.45	0.39±0.92	0.74±1.02	0.51±1.11	1.09±1.15

Relative organ weight was calculated as (organ weight (g)/body weight of the animal on sacrifice day (g)) × 100. Values are expressed as mean ± SD (n=3). There were no significant differences in the relative organ weight among different groups (p>0.05).

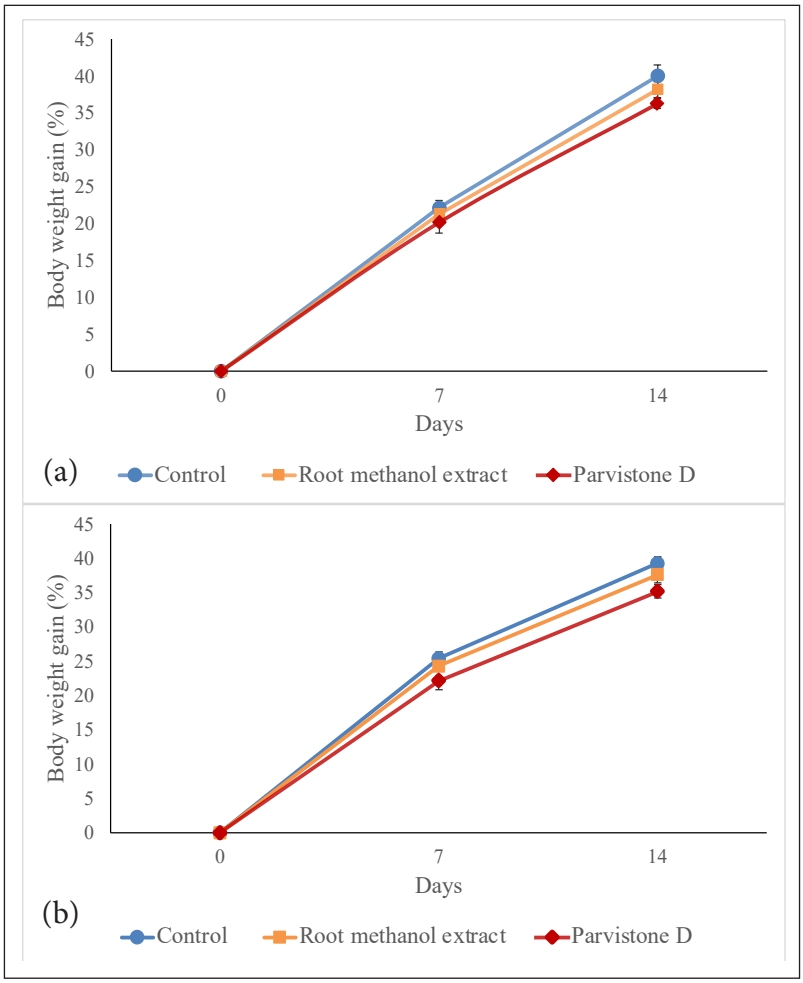

Fig. 1. Effects of root methanol extract and parvistone D on the percentage of body weight in male (*a*) and female (*b*) mice during a 14-day oral acute toxicity study. Values are expressed as mean ± SD (n=3).

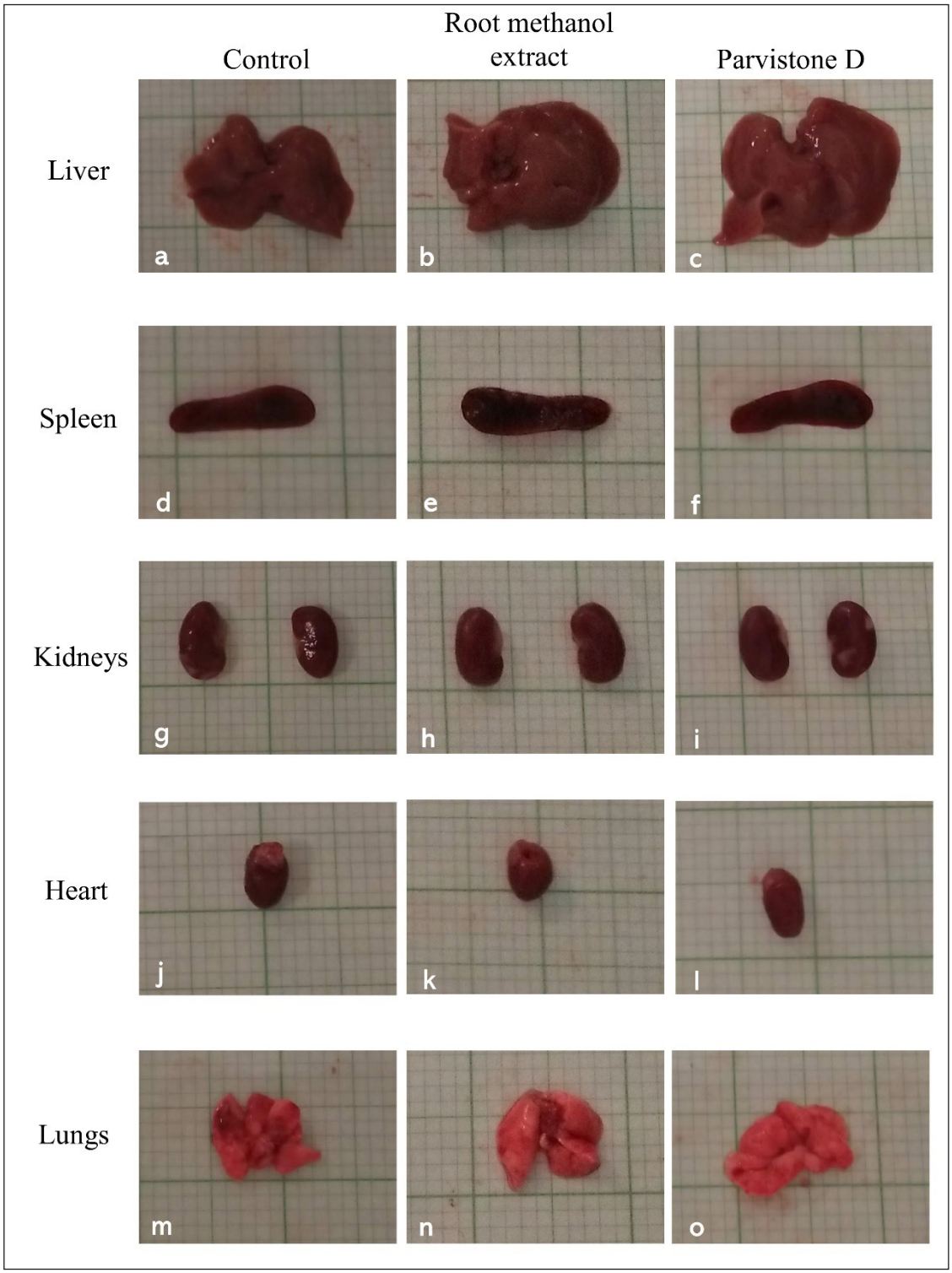

Fig. 2. Gross appearance of liver (a-c), spleen (d-f), kidneys (g-i), heart (j-l), and lungs (m-o) of male mice from control, root methanol extract, and parvistone D treated groups showing normal morphology.

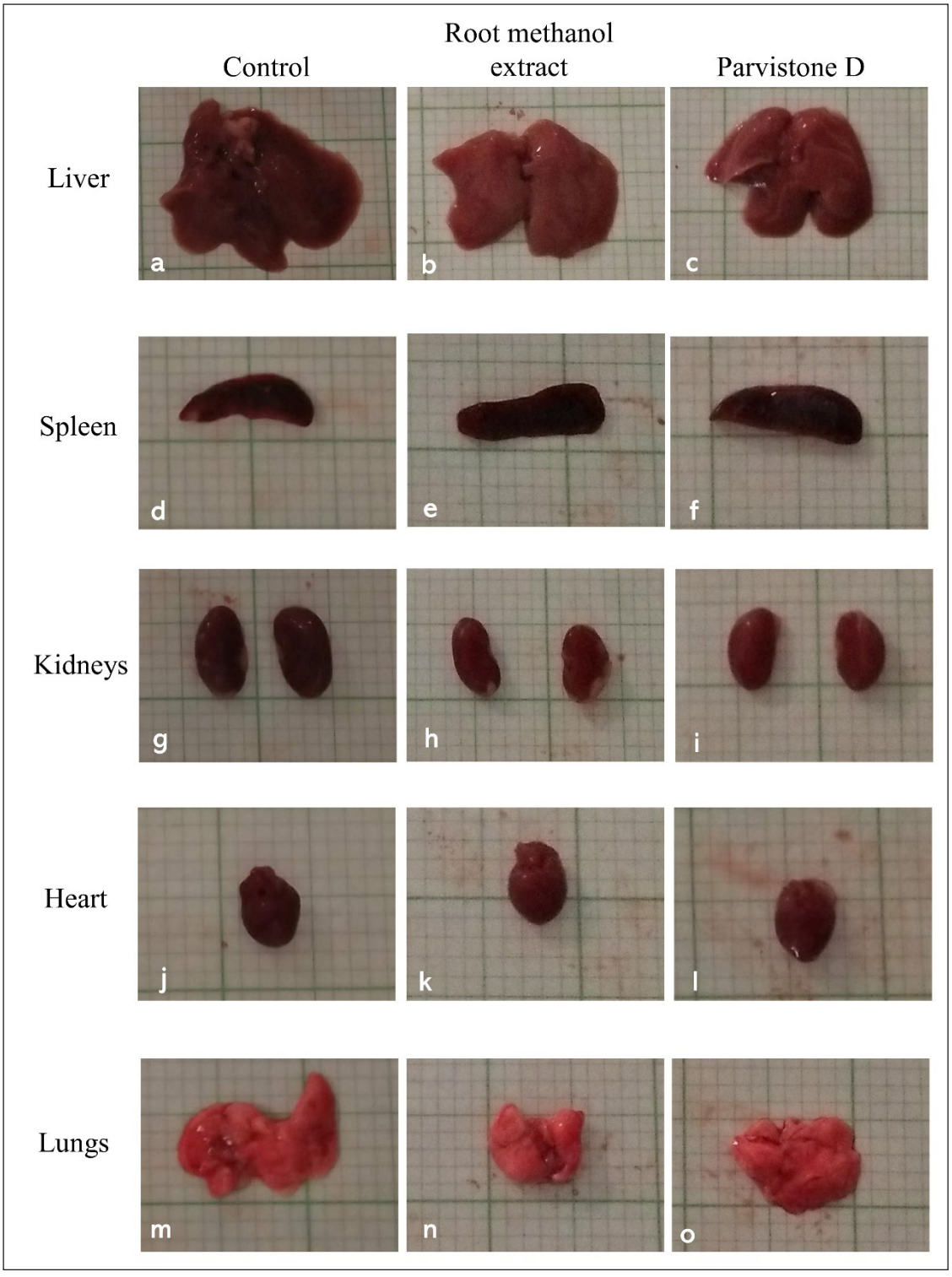

Fig. 3. Gross appearance of liver (a-c), spleen (d-f), kidneys (g-i), heart (j-l) and lungs (m-o) of female mice from control, root methanol extract and parvistone D treated groups showing normal morphology.

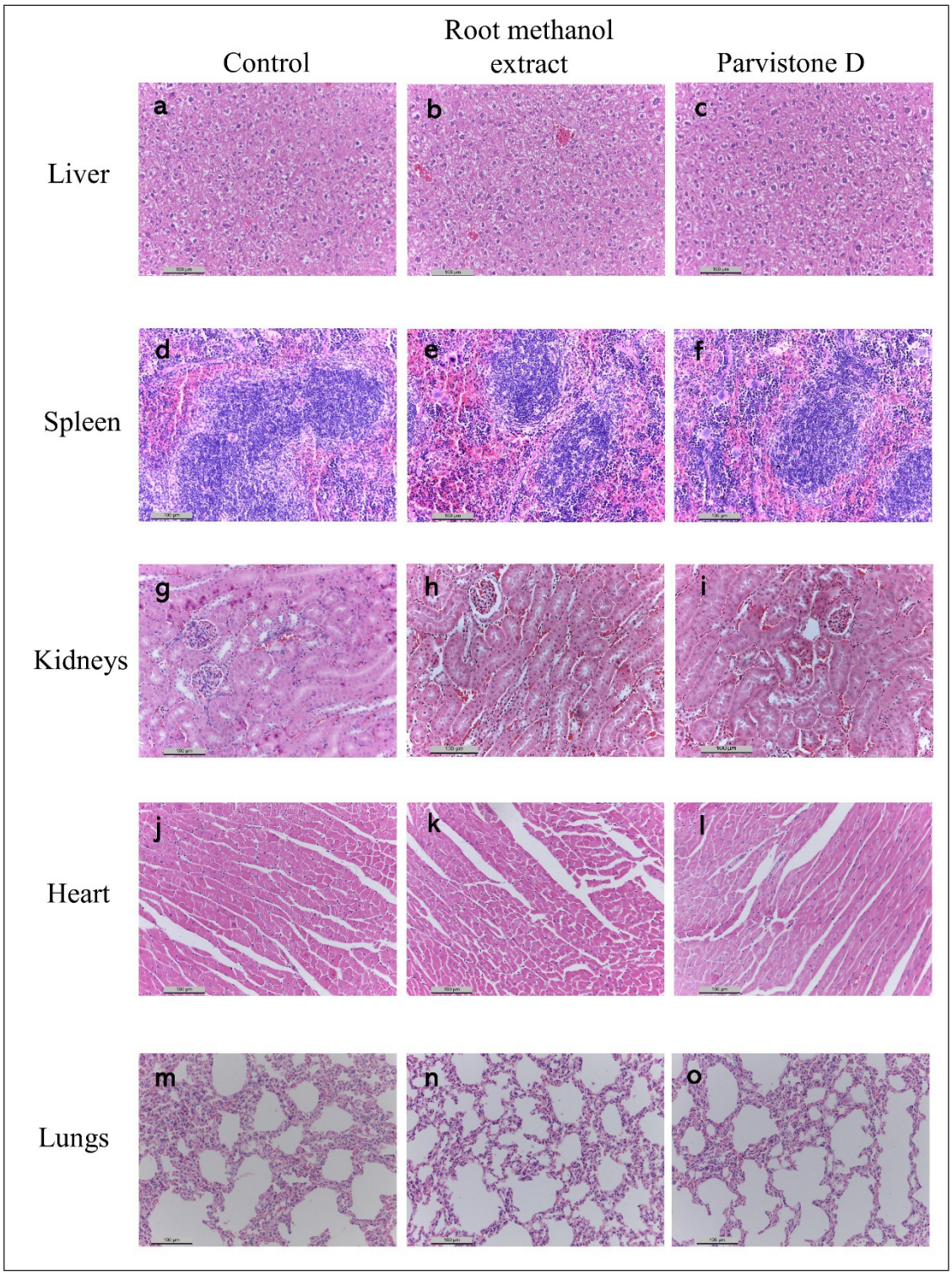

Fig. 4. Representative photomicrographs of liver (a-c), spleen (d-f), kidneys (g-i), heart (j-l) and lungs (m-o) tissue sections of male mice in acute oral toxicity study (H&E stain, x200). No pathological changes were detected in control, root methanol extract and parvistone D treated mice.

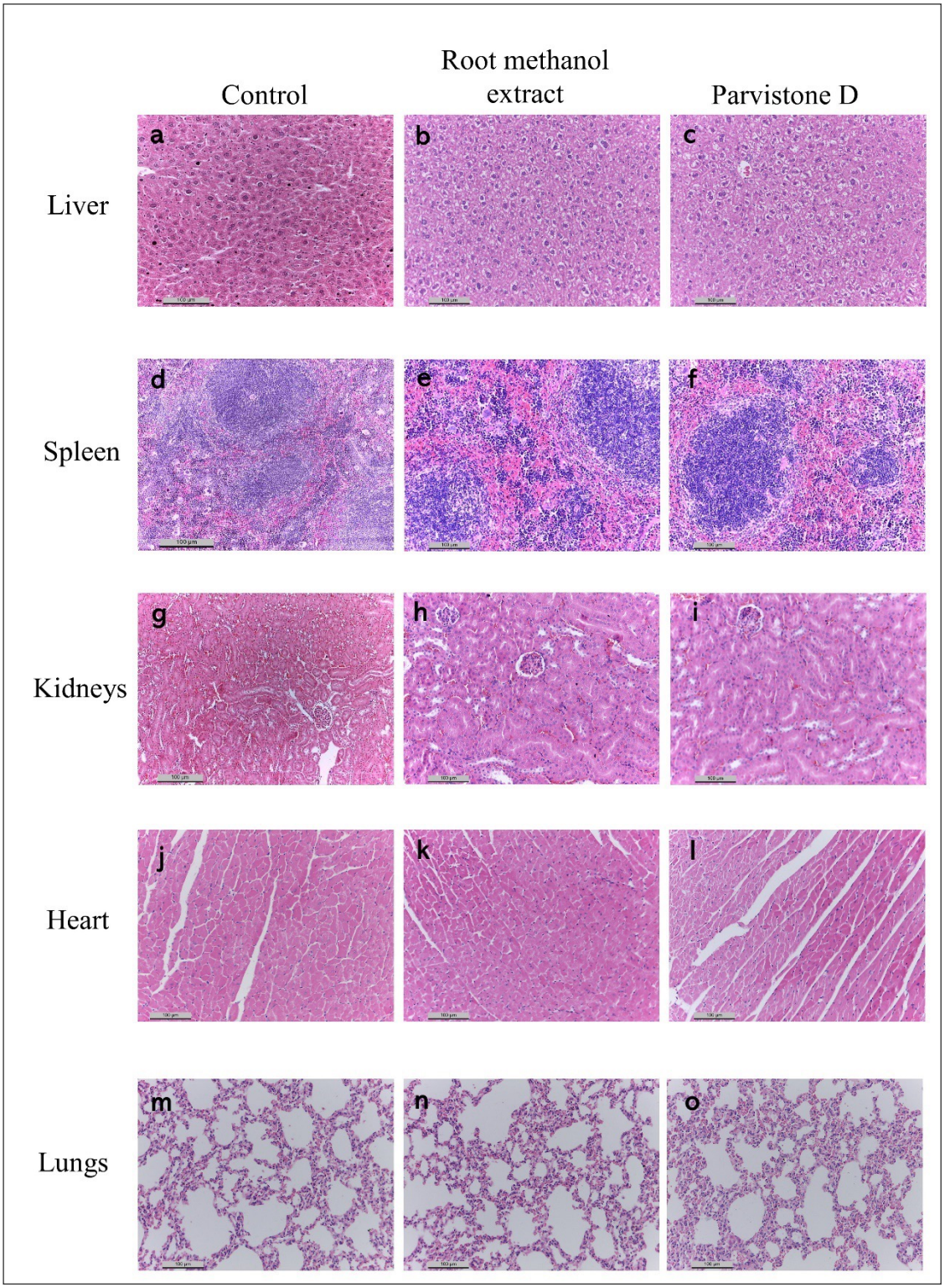

Fig. 5. Representative photomicrographs of liver (a-c), spleen (d-f), kidneys (g-i), heart (j-l) and lungs (m-o) tissue sections of female mice in acute oral toxicity study (H&E stain, x200). No pathological changes were detected in control, root methanol extract and parvistone D treated mice.

CONCLUSION

In conclusion, the root methanol extract, and parvistone D of *G. lanceolatus* are relatively nontoxic, and the no-observed-adverse-effect level (NOAEL) of the extract and compound was determined as 2000 and 500 mg/kg bwt, respectively. Nevertheless, further toxicity testing such as subacute and chronic toxicity studies using repeated doses of the extract, and compound should be performed to ascertain its safety on prolonged use.

ACKNOWLEDGEMENTS

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ETHICAL STATEMENT

All animal procedures were conducted in conformity with National and International Guidelines for Handling of Laboratory Animals with approval from the UiTM Animal Research and Ethics Committee (103/2015).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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