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Subchronic toxicity study of the ethanol root extract of *Globba pendula* Roxb. In Vivo experimental models

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ABSTRACT

Globba pendula Roxb has a long history of use by the Khmer people and residents of the Mekong Delta for treating musculoskeletal pain, arthritis, and chronic inflammatory diseases. Preliminary studies indicate that the ethanol extract from its roots contains high levels of phenolics and flavonoids, exhibits strong antioxidant activity, and demonstrates selective cytotoxicity against certain cancer cell lines. However, the subchronic toxicity of this extract has not yet been evaluated in an in vivo model. To evaluate the subchronic toxicity of the ethanol root extract of *Globba pendula* Roxb. in mice. The ethanol root extract of *Globba pendula* Roxb. was collected from Dinh Mountain (Ba Ria – Vung Tau) and authenticated at the Institute of Tropical Biology, Ho Chi Minh City. The study was conducted on male and female Swiss albino mice. The mice were orally administered the extract at doses of 500 mg/Kg and 1000 mg/Kg/day for 28 consecutive days. Body weight, behavior, spontaneous locomotor activity, hematological parameters, and serum biochemistry (AST, ALT, creatinine, urea) were monitored. Gross and histopathological examinations of the liver and kidneys were also performed. The subchronic toxicity test at doses of 500 mg/Kg and 1000 mg/Kg over 28 days revealed no signs of toxicity in the experimental animals. The extract did not affect physical growth, locomotor behavior, hematological indices, or biochemical functions of the liver (AST, ALT) and kidneys (urea, creatinine) compared to the control group. Furthermore, gross and

*histopathological analyses confirmed that the liver and kidney parenchymal structures were preserved intact, with no observed inflammation, necrosis, or degeneration. The results indicate that the ethanol root extract of *Globba pendula* Roxb. did not exhibit abnormal toxicity in the subchronic toxicity model in experimental animals.*

1. INTRODUCTION

Globba pendula Roxb. (Zingiberaceae) is a traditional medicinal herb with a long history of use by the Khmer people and residents of the Mekong Delta for the treatment of musculoskeletal pain, arthritis, and chronic inflammatory diseases (Williams, 2004; Võ Văn Chi, 1991). Our preliminary studies indicated that the ethanol root extract possesses high total phenolic and flavonoid contents, exhibits potent antioxidant activity (low DPPH IC₅₀ values), and demonstrates selective cytotoxicity against several human cancer cell lines while showing minimal toxicity to normal cells.

To develop this extract into health supplements or therapeutic adjuncts, the evaluation of toxicity, particularly subchronic toxicity (28 days) in *in vivo* models, is a mandatory step to ensure safety for prolonged use (Aslam et al., 2017; Chanda et al., 2009).

Consequently, this study was conducted to evaluate the subchronic toxicity of the ethanol root extract of *Globba pendula* Roxb. in Swiss albino mice, thereby providing a scientific basis for the future application of this medicinal plant.

2. MATERIALS AND METHODS

2.1 Plant material and extract preparation

The rhizomes of *Globba pendula* Roxb. were collected at Dinh Mountain, Vung Tau, and

formally identified at the Institute of Tropical Biology in Ho Chi Minh City.

The standardized ethanol root extract of *Globba pendula* Roxb. was prepared using the following protocol: dried roots were ground into powder and subjected to ultrasonic-assisted extraction with 96% ethanol (drug-to-solvent ratio of 1:20) at 40°C for 90 minutes, repeated three times. The combined extracts were concentrated under reduced pressure using a rotary evaporator at 50°C to obtain a crude extract.

2.2 Experimental animals

Male and female Swiss albino mice (4–5 weeks old, weighing 20–25 g) were supplied by the Pasteur Institute, Ho Chi Minh City. The animals were acclimatized for 7 days prior to the experiment at the Experimental Animal Research Unit, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy. The study was conducted in compliance with the OECD Guideline 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents) and the "Guidelines for Preclinical and Clinical Trials of Traditional Medicines and Herbal Drugs" issued by the Ministry of Health (Ministry of Health of Vietnam, 2015; OECD, 2008).

2.3 Chemicals and equipment

Kits for hematological and biochemical quantification, as well as chemicals for Hematoxylin and Eosin (H&E) staining, met research standards. Equipment included an automated hematology analyzer, a biochemistry analyzer, and an optical microscope.

2.4 Research methods

The doses used in the subchronic toxicity study were selected in accordance with OECD guideline 407 (2008) and Decision No. 141/QĐ-K2ĐT issued by the Ministry of Health of Vietnam. Dose selection was based on available acute toxicity data and relevant previous studies on the genus *Globba*.

Preliminary acute toxicity assessments and published data indicate that the extract of *Globba pendula* L. roots exhibits very low toxicity. In a study by Ha et al. (2021), oral administration of a single maximum feasible dose (limit dose of 10,000 mg/Kg body weight) in mice did not result in mortality or observable signs of toxicity. As the median lethal dose (LD₅₀) could not be determined, dose selection for the subchronic toxicity study followed the limit test approach (WHO, 2020).

Accordingly, 1000 mg/Kg body weight was selected as the high dose to evaluate the safety margin of the extract during repeated administration. A lower dose of 500 mg/Kg body weight (equivalent to half of the high dose) was included to assess potential dose–response relationships in hematological, biochemical, and histopathological parameters. Notably, the dose of 500 mg/Kg has previously been reported to exert significant anti-inflammatory and antioxidant activities in *in vivo* models (Ha et al., 2021).

Experimental design: A subchronic toxicity study with a randomized, controlled design. The mice were randomly divided into 4 groups (n = 10/group, 5 males + 5 females):

Group 1: Physiological control (distilled water, PO)

Group 2: Vehicle control (DMSO, PO)

Group 3: Extract dose 500 mg/Kg/day, PO

Group 4: Extract dose 1000 mg/Kg/day, PO

The extract was suspended in distilled water and administered orally via gastric gavage once daily for 28 consecutive days.

During the experiment, mice were monitored daily for clinical signs and behavioral changes. Body weight was recorded weekly to assess growth. Spontaneous locomotor activity was evaluated weekly (on days 1, 7, 14, and 28) following the method described by Obese (Obese et al., 2021). Mice were placed in the testing chamber for a 1-minute acclimatization period. After a 30-minute rest, the formal test was conducted by placing each individual into the chamber at a fixed position. The number of horizontal and vertical movements was recorded over a 2-minute period.

On day 29, mice were anesthetized with chloroform, and blood samples were collected via cardiac puncture for hematological analysis (white blood cells, red blood cells, platelets, hemoglobin, and hematocrit) and serum biochemistry (AST, ALT, blood urea, and serum creatinine). Subsequently, the liver and kidneys were harvested for gross examination and histopathological analysis (H&E staining).

2.5 Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 20.0 software, employing

One-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effect of the ethanol root extract of *Globba pendula* Roxb. on mice body weight

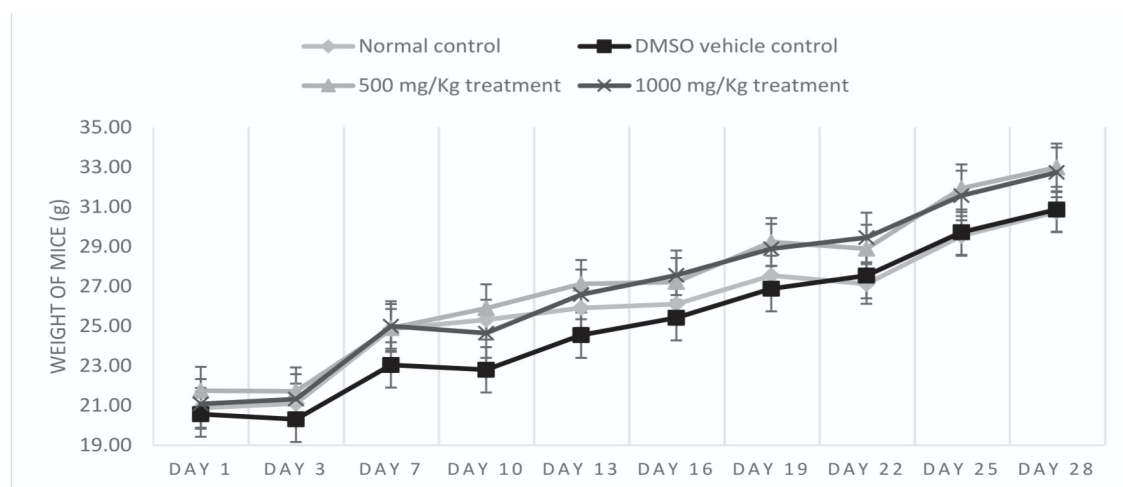


Figure 1. Mice body weight in the physiological control, vehicle control (DMSO), 500 mg/Kg, and 1000 mg/Kg treatment groups.

The body weight of mice in all groups gradually increased over time (from an initial 22.5 ± 1.2 g to approximately 28–30 g after 28 days). There were no statistically significant differences between the physiological control (29.8 ± 1.4 g), vehicle control (29.5 ± 1.3 g), 500

mg/Kg (29.2 ± 1.5 g), and 1000 mg/Kg (28.9 ± 1.4 g) groups ($p > 0.05$).

3.2 Effect of *Globba pendula* Roxb. ethanol root extract on spontaneous locomotor activity in mice

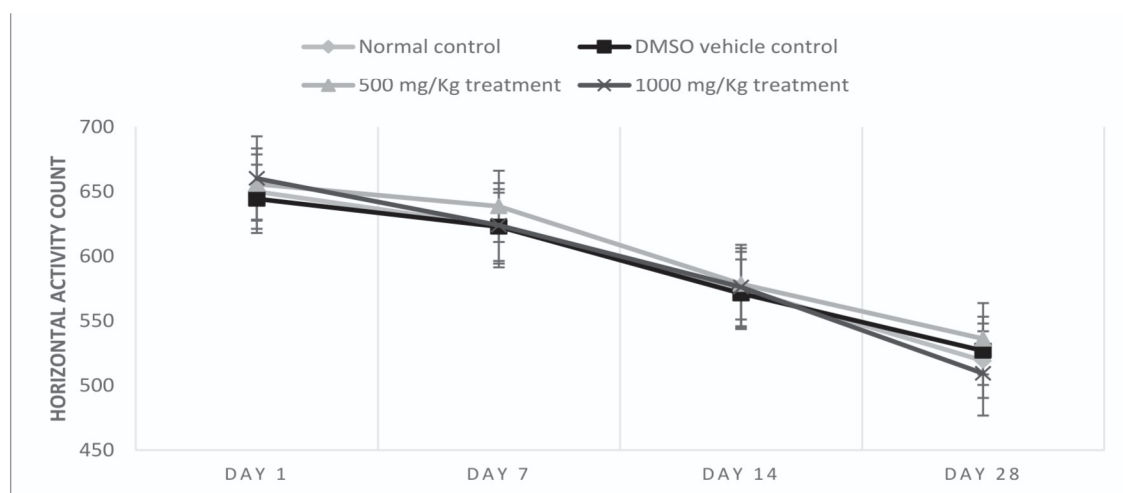


Figure 2. Number of horizontal movements of mice in the physiological control, vehicle control (DMSO), and experimental groups (500 mg/Kg and 1000 mg/Kg) in the spontaneous locomotor activity model

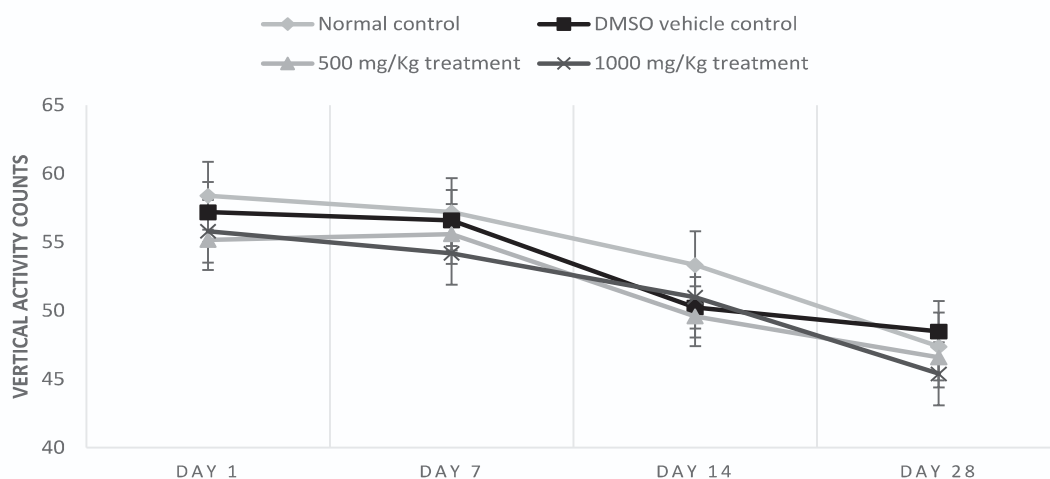


Figure 3. Number of vertical movements of mice in the physiological control, vehicle control (DMSO), and treatment groups (500 mg/Kg and 1000 mg/Kg) in the spontaneous locomotor activity model

The results from Figures 2 and 3 show that the number of horizontal and vertical movements in the treatment groups (500 mg/Kg: horizontal 45 ± 5 , vertical 20 ± 3 ; 1000 mg/Kg: horizontal 43 ± 4 , vertical 19 ± 3) did not differ significantly from those of the physiological control (horizontal 48

± 5 , vertical 22 ± 3) and vehicle control (DMSO) (horizontal 46 ± 4 , vertical 21 ± 3) ($p > 0.05$)

3.3 Effect of Globba pendula Roxb. ethanol root extract on hematological parameters in mice

Table 1. Hematological parameters of mice in the physiological control, vehicle control (DMSO), and treatment groups (500 mg/Kg and 1000 mg/Kg) at the end of the experiment.

Group	Normal control	DMSO vehicle control	500 mg/Kg treatment	1000 mg/Kg treatment
WBC ($10^9/L$)	$9,74 \pm 1,30$	$9,80 \pm 1,98$	$9,87 \pm 1,11$	$9,83 \pm 1,05$
RBC ($10^{12}/L$)	$9,40 \pm 0,47$	$9,47 \pm 0,91$	$9,55 \pm 0,54$	$9,86 \pm 0,66$
HGB (g/dL)	$136,00 \pm 7,38$	$132,40 \pm 9,69$	$144,20 \pm 9,63$	$155,60 \pm 11,08$
HCT (%)	$42,72 \pm 2,45$	$41,38 \pm 1,64$	$44,62 \pm 1,62$	$45,16 \pm 1,53$
PLT ($10^3/L$)	$727,20 \pm 104,27$	$757,80 \pm 111,50$	$796,80 \pm 115,28$	$740,40 \pm 142,32$

Hematological parameters (white blood cells: $8-10 \times 10^9/L$, red blood cells: $7-9 \times 10^{12}/L$, hemoglobin: $120-140$ g/L, etc.) in the treatment

groups showed no statistically significant differences compared to the control groups ($p > 0,05$).

3.4 Effect of *Globba pendula* Roxb. ethanol root extract on serum biochemical parameters in mice

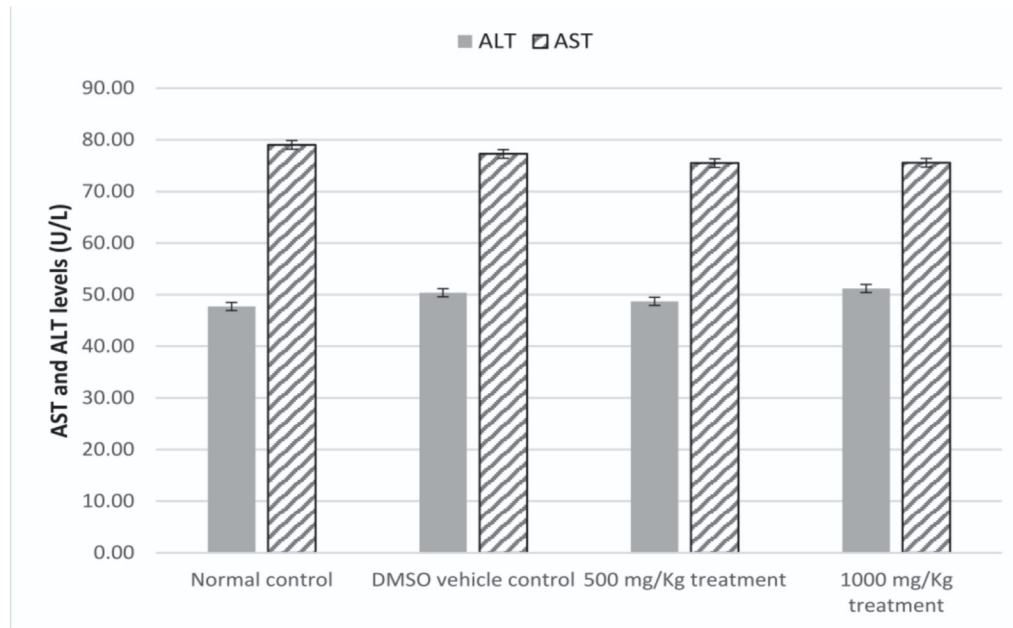


Figure 4. AST and ALT levels of mice in the physiological control, vehicle control (DMSO), and treatment groups (500 mg/Kg and 1000 mg/Kg) at the end of the experiment

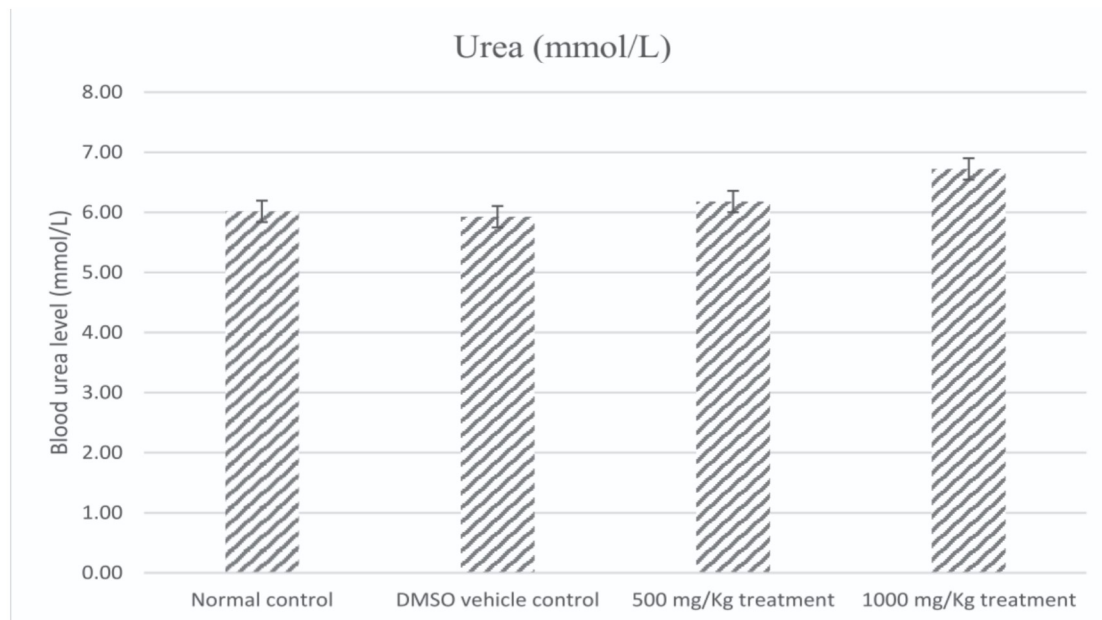


Figure 5. Blood urea levels of mice in the physiological control, vehicle control (DMSO), and treatment groups (500 mg/Kg and 1000 mg/Kg) at the end of the experiment

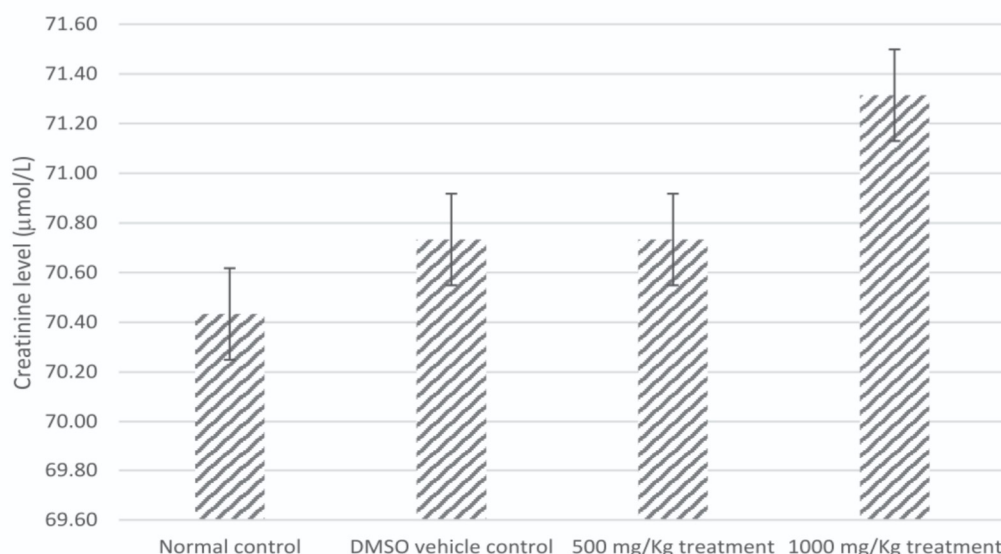


Figure 6. Creatinine levels of mice in the physiological control, vehicle control (DMSO), and treatment groups (500 mg/Kg and 1000 mg/Kg) at the end of the experiment.

The AST/ALT (80–100 U/L), urea (5–7 mmol/L), and creatinine (40–50 μmol/L) levels in the treatment groups showed no statistically significant differences compared to the control groups ($p > 0.05$).

3.5 Effect of *Globba pendula* Roxb. ethanol root extract on mice liver and kidneys histopathology

Macroscopic morphology of mice liver and kidney

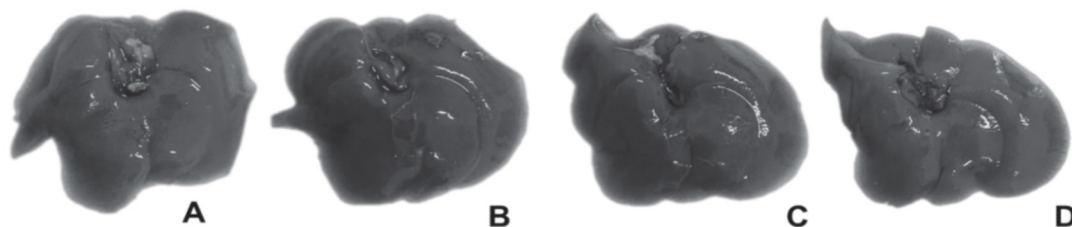


Figure 7. Gross morphology of mouse livers in the experimental groups: (A) Physiological control; (B) Vehicle control (DMSO); (C) Extract dose 500 mg/Kg; (D) Extract dose 1000 mg/Kg

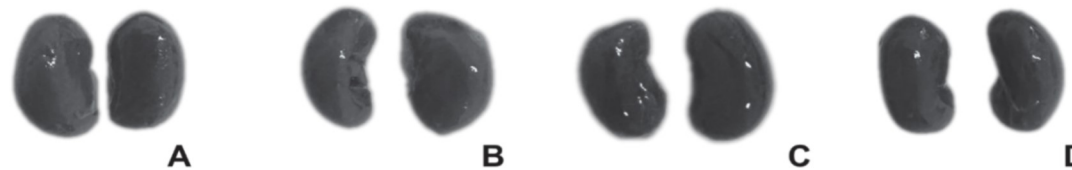


Figure 8. Gross morphology of mouse kidneys in the experimental groups: (A) Physiological control; (B) Vehicle control (DMSO); (C) Extract dose 500 mg/Kg; (D) Extract dose 1000 mg/Kg.

The results presented in Figures 7 and 8 demonstrate that oral administration of the extract

at doses of 500 mg/Kg and 1000 mg/Kg for 28 days caused no gross abnormalities in the liver

and kidneys. The organs exhibited a normal bright red color and smooth surface, with no signs of edema, congestion, or hypertrophy, comparable to the physiological and vehicle

Microscopic morphology of mice liver and kidneys

control groups. Both doses maintained normal gross morphology without any indications of surface damage, such as hemorrhage or surface roughness.

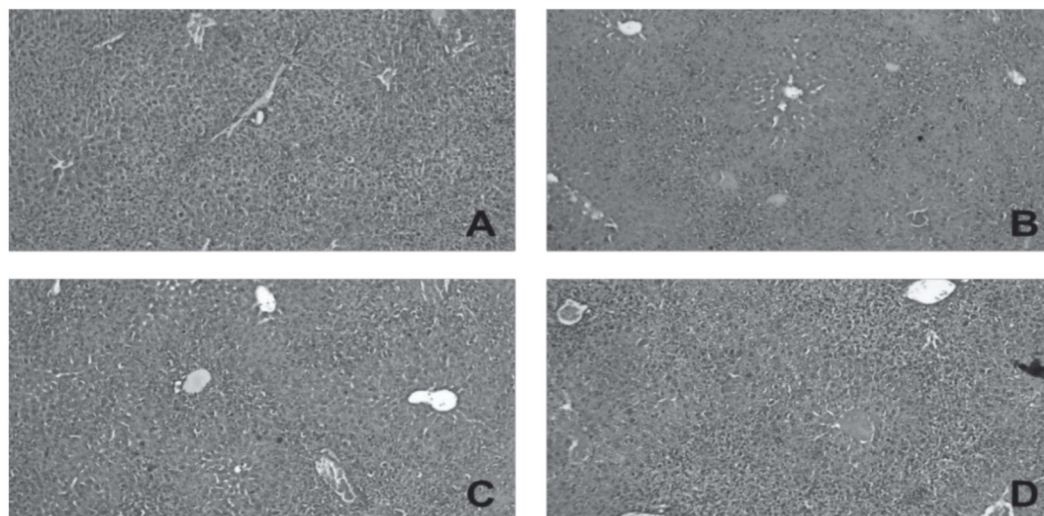


Figure 9. Histopathology of mouse liver tissues in the experimental groups: (A) Physiological control; (B) Vehicle control (DMSO); (C) Extract dose 500 mg/Kg; (D) Extract dose 1000 mg/Kg.

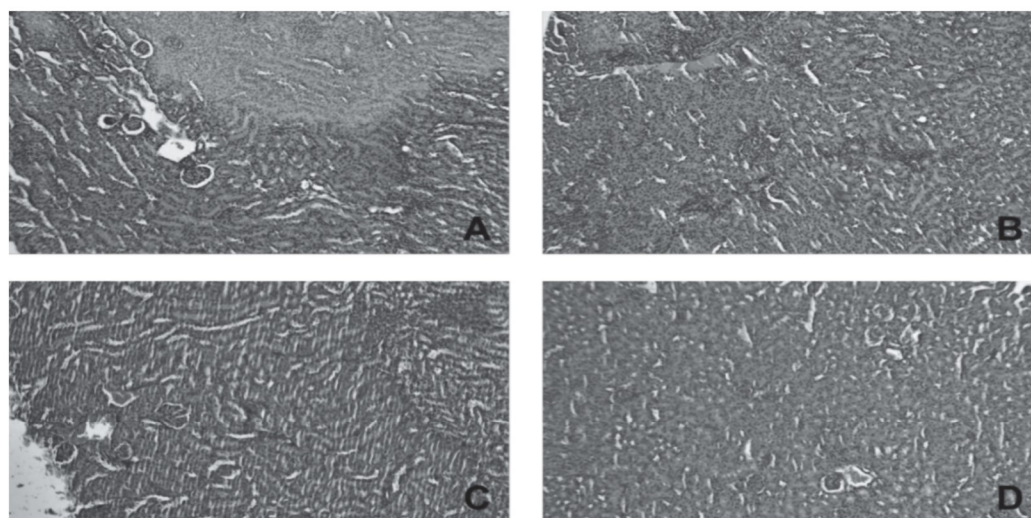


Figure 10. Histopathology of mouse kidney tissues in the experimental groups: (A) Physiological control; (B) Vehicle control (DMSO); (C) Extract dose 500 mg/Kg; (D) Extract dose 1000 mg/Kg

The results presented in Figures 9 and 10 show that the sinusoidal architecture and cellular structures of the liver and kidneys remained

intact, with no signs of inflammation or necrosis in the extract-treated groups. Specifically, hepatocytes appeared uniform in size, central

veins were not congested, and portal tracts showed no inflammation. Similarly, glomeruli were regular in size, the interstitium was free of inflammation, and renal tubules were uninjured. These features were comparable to the physiological and vehicle control (DMSO) groups. Both doses maintained normal microscopic structure without fatty degeneration or inflammatory infiltration.

The 28-day subchronic toxicity study demonstrated that the ethanol extract of *Globba pendula* Roxb. roots administered at doses of 500 and 1000 mg/Kg exhibited a favorable safety profile, as no mortality or apparent clinical signs of toxicity were observed. These findings are consistent with recent toxicological evaluations of other members of the Zingiberaceae family. A review by Nuari et al. (2023) reported that *Globba pendula* and related species were classified as non-toxic in both acute and subacute toxicity models, indicating that their characteristic phytochemicals, including phenolates and benzofurans, do not induce systemic toxicity at comparable exposure levels. Similarly, extracts of *Zingiber zerumbet* (Chan et al., 2024) and *Zingiber roseum* (Amanat et al., 2024) have been shown to be well tolerated during prolonged administration, without causing significant adverse effects on general health status or gross morphology.

The absence of changes in body-weight gain and spontaneous locomotor activity in both treatment groups suggests that the extract did not adversely affect basal metabolic processes or central nervous system function. This observation is consistent with previous reports on *Zingiber officinale*, in which doses up to 1000

mg/Kg were considered safe (Benny et al., 2021). Although minor alterations in body weight or behavior have been reported in certain Zingiber species at very high doses (≥ 1000 –2000 mg/Kg) (Chang et al., 2012; Yakubu et al., 2024), such effects were not observed in the present study, indicating that *G. pendula* remained within a non-toxic dose range under the experimental conditions.

Hematological parameters, including white blood cell count (WBC), red blood cell count (RBC), and hemoglobin concentration, remained within normal physiological ranges, indicating that the extract did not induce myelotoxicity or disrupt erythropoiesis. Consistent with these findings, serum biochemical markers of hepatic and renal function - namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine - did not differ significantly from those of the control group, suggesting preserved liver and kidney function. Given that the liver and kidneys play central roles in the metabolism and excretion of herbal constituents, the absence of functional impairment in these organs is particularly important for safety evaluation. The favorable safety profile observed may be partly attributable to the high content of phenolic and flavonoid compounds in the extract. A study by Amanat et al. (2021) on *Zingiber roseum* demonstrated that these polyphenolic compounds are not only non-toxic but also exert antioxidant effects, thereby protecting the liver against oxidative stress-induced injury.

Histopathological analysis further supported the biochemical findings, as liver and kidney tissues from treated animals preserved normal

architecture without evidence of inflammation, necrosis, or degenerative changes. The maintenance of histological integrity indicates that *G. pendula* does not exert adverse effects at the cellular level following repeated administration. This characteristic is a prerequisite for the potential therapeutic application of the plant, particularly in the management of inflammatory diseases. Notably, selinene - identified by Phuong et al. (2020) as a major constituent of *G. Pendula* - has been reported to exhibit moderate nitric oxide (NO) inhibitory activity and anti-inflammatory properties.

In conclusion, the results of this 28-day subchronic toxicity study provide experimental evidence supporting the safe use of *Globba pendula* Roxb. at doses up to 1000 mg/Kg. The absence of treatment-related toxicity reinforces the scientific basis for its traditional use by the Khmer community in the management of chronic inflammatory conditions and suggests a relatively wide safety margin. These findings provide a foundation for further investigations, including longer-term toxicity studies and clinical evaluations, to support the development of *G. pendula*-based therapeutic preparations.

5. CONCLUSION

The subchronic toxicity study in mice indicated that the ethanol root extract of *Globba pendula* Roxb., administered orally at doses of 500 mg/Kg and 1000 mg/Kg for 28 consecutive days, is safe and did not induce any signs of toxicity in experimental animals. Throughout the 28-day experimental period, mice in the extract-treated groups exhibited normal weight gain and showed no abnormalities in behavior or

locomotor activity compared to the control group ($p > 0.05$). Hematological and serum biochemical parameters (AST, ALT, urea, creatinine) remained within normal physiological limits and showed no statistically significant differences from the control group ($p > 0.05$). Furthermore, gross examination and H&E-stained histopathological analysis confirmed that the liver and kidney structures were preserved intact, with no evidence of cellular injury, inflammation, or necrosis. These results demonstrate the high safety profile of the medicinal material, providing a reliable scientific basis for the development and application of *Globba pendula* root-based products for long-term therapeutic support.

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