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RESEARCH ARTICLE

Acute oral toxicity and dose-dependent histopathological effects of Indonesian *Ciplukan* (*Physalis angulata* L.) extract in Sprague-Dawley rats

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Abstract

The growing global interest in herbal medicines underscores the importance of safety assessments for traditional plants. *Physalis angulata* L. (*Ciplukan*) demonstrates therapeutic potential, yet data on Indonesian extracts remain limited. This study evaluated the acute oral toxicity and determined the median lethal dose (LD_{50}) of Indonesian *Ciplukan* extract. Twenty-five female Sprague-Dawley rats were divided into five groups (n=5): control (aqueous), and four groups that received extract at 100, 400, 800, or 1200 mg/kg body weight. Over 14 days, researchers monitored clinical signs, body weight, food intake, and organ weights in accordance with Acute Oral Toxicity (OECD Test Guideline 425). Major organs were examined histopathological using the modified International Harmonization of Nomenclature and Diagnostic Criteria. No mortality or significant behavioral changes were observed at any dose. Rats maintained normal body weight gain and food intake. The LD_{50} was determined to be exceeded 1200 mg/kg, indicating favourable acute safety. However, histopathological analysis revealed significant dose-dependent necrotic changes ($p < 0.05$) in the lungs, kidneys, liver, and spleen at doses of 400 mg/kg and above. Liver and spleen damage were detected first at this threshold. These findings indicate an LD_{50} above 1200 mg/kg body weight for Indonesian *Ciplukan* extract, supporting its acute safety. Nonetheless, subclinical organ toxicity occurred at doses of 400 mg/kg or higher, emphasizing the need for dose optimization in phytomedicine. The study provides regulatory-grade toxicological data to support evidence-based standardization of Indonesian herbal medicines and highlights the necessity for further research on sub chronic and chronic toxicity to establish safe therapeutic doses.

1. INTRODUCTION

The global resurgence of interest in herbal medicines highlights the need for comprehensive safety assessments of traditionally used plants, particularly in biodiversity-rich countries such as Indonesia, where herbal medicine has long been an integral to healthcare (1,2). Indonesia boasts remarkable biodiversity, with approximately 30,000 plant species, nearly 7,000 of which have documented medicinal value (3). Among these, *Physalis angulata* L. (locally known as *Ciplukan*), a member of the Solanaceae family, has garnered attention for its therapeutic potential. Traditionally used for various

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ailments, recent studies confirm its anti-inflammatory, antimicrobial, and antineoplastic properties (4). Notably, *P. angulata* extract has demonstrated efficacy in improving both macroscopic and microscopic inflammatory features in a psoriasis-like mouse model induced with 5% imiquimod cream, suggesting its potential as an alternative treatment for psoriasis (5). Additionally, research shows the extract effectively ameliorates skin fibrosis in scleroderma patients, broadening its possible applications in managing chronic inflammatory skin disorders (6). Furthermore, *P. angulata* leaf extract has been shown to alleviate atopic dermatitis in mice by normalizing Interleukin (IL)-4 levels, demonstrating its immunomodulatory properties in allergic skin disorders (7).

Despite its pharmacological potential, comprehensive toxicological data on Indonesian *Ciplukan* extracts remain limited, particularly regarding their acute oral toxicity. The phenotype and safety profiles of medicinal plants can vary due to environmental and genetic factors (8), making it essential to assess the specific risks associated with the Indonesian variety of *Ciplukan*. Therefore, focused evaluation of this local variant is warranted to ensure the safety of its local use and potential development into standardized phytomedicines. Acute oral toxicity studies play a crucial role in evaluating the immediate adverse effects of natural products at high doses, providing essential data for regulatory approval, clinical applications, and safe dosage determination (9), Understanding the dose-dependent effects of *Ciplukan* extract is fundamental to its development as a standardised phytomedicine, ensuring both safety and efficacy for broader therapeutic use.

This study represents the first acute toxicity evaluation of *P. angulata* L. from Mersi Village, Banyumas Regency, Central Java, Indonesia, addressing two critical gaps. First, phytochemical profiles vary with environmental factors and the unique tropical conditions of Banyumas may influence physalin concentrations differently than in other regions. Second, this research provides the first systematic histopathological examination of five vital organs using modified INHAND criteria with quantitative scoring. By assessing the extract's safety profile and median lethal dose (LD_{50}) through internationally recognized toxicity testing guidelines, specifically the Organization for Economic Co-operation and Development (OECD) Test Guideline No. 425 for Acute Oral Toxicity, this study aims to support the evidence-based development of Indonesian herbal medicines, bridging traditional knowledge with rigorous scientific validation (10).

2. MATERIALS AND METHODS

2.1. Plant Material and Extract Preparation

Fresh *P. angulata* L. (*Ciplukan*) plants were collected from Mersi, South Purwokerto Regency, Indonesia, and authenticated by a botanist from the Faculty of Biology, Jenderal Soedirman University, Indonesia (certificate no. B/799/UN23.6.10/DI/2020). The aerial parts were cleaned, air-dried at room temperature, and ground into a fine powder. Based our previous study (5), which identified ethyl acetate as optimal solvent for extracting physalins B, D, and F, from *Ciplukan* extract, this method to ensure consistency and focus the present work on the pharmacologically relevant fraction. The extract was filtered, concentrated using a rotary evaporator at 50°C, and stored at 4°C until further use. For administration, the ethyl acetate fraction was dissolved in an aqueous solution (1 mL per dose) and administered via oral gavage. The extract was given as a single dose for acute oral toxicity evaluation.

2.2. Experimental Animals

Twenty-five female Sprague-Dawley rats (8-12 weeks old, weighing 150-200 g) were obtained from the Experimental Animal Development Unit, Yogyakarta, Indonesia. Female rats were chosen due to their greater sensitivity to toxicants and to aligns with OECD Test Guideline No. 425 recommendations. Although rats share many physiological and behavioral similarities with humans, certain anatomical differences exist, such as direct esophageal connection to the stomach that prevents regurgitation and the absence of a gallbladder (11).

The rats were randomly assigned to five groups (n=5 per group): Group 1 (G1) served as the control and received the vehicle (aqueous solution) only, while Groups 2-5 (G2-G5) received single doses of *Ciplukan* extract at 100, 400, 800, and 1200 mg/kg body weight, respectively. Doses were selected based on our previous efficacy study (5) using 400-1200 mg/kg, with additional 100 mg/kg dose included to establish a safety margin between therapeutic and potentially toxic levels in accordance with National Agency of Drug and Food Control (BPOM) Regulation No. 10/2022.

Rats were housed in polypropylene cages (3-4 per cage) under standard laboratory conditions (temperature 22 ± 3°C, relative humidity 55 ± 5%, and a 12-hour light/dark cycle). A standard pellet diet and water were provided *ad libitum*. Inclusion criteria included healthy rats with normal anatomy, non-pregnant and nulliparous status, and body weight within ±20% of the average weight of all experimental animals. The rats were acclimatized for one week prior to the experiments. All procedures were approved by the Health Research Ethics Committee, Faculty of Medicine, Jenderal Soedirman University (approval no. 039/KEPK/PE/VI/2022) and conducted in compliance with BPOM Regulation No. 7 of 2014 and OECD Test Guideline No. 425 for Acute Oral Toxicity.

2.3. Acute Oral Toxicity Study

The acute oral toxicity study was conducted in accordance with BPOM Regulation No. 10 of 2022, which aligns with OECD Test Guideline No. 425 and specifies a 14-day observation period for acute oral toxicity assessment (12). The regulation outlines key evaluation parameters, including the median LD_{50}, behavior, body weight monitoring, and pathological examination of vital organs. Female rats were fasted for 14–18 hours prior to extract administration (with free access to water) and continued fasting for 1–2 hours post-dosing. The extract was administered via oral gavage. Animals were observed continuously for 24 hours post-administration, with particular attention during the first 4 hours, and subsequently monitored daily for 14 days.

Observations included changes in skin and fur, eyes and mucous membranes, respiratory and circulatory function, autonomic and central nervous systems activity, and somatomotor coordination and behavior pattern. Specific symptoms such as tremors, convulsions, salivation, diarrhea, lethargy, sleep disturbances, or coma were carefully recorded (12).

Body weight was measured before dosing (day 0), and again on days 7 and 14 using a calibrated digital scale (Model OHAUS PX2202, OHAUS Corporation). Measurements were taken at the same time of day between 9:00 and 10:00 AM under fasting conditions to minimize variability. A body weight change exceeding 20% from baseline was considered indicative of potential toxicity, in accordance with OECD toxicity testing guidelines. Daily food consumption was also recorded during the study period. On day 15, all animals were euthanized by CO_2 asphyxiation, followed by a complete gross necropsy. Major organs, including the liver, kidneys, heart, lungs, and spleen, were excised, weighed, and examined macroscopically. The organs were then fixed in 10% neutral buffered formalin, processed using standard histological procedures, embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E) for microscopic examination.

Histopathological examination of the heart was conducted using a modified version of the International Harmonization of Nomenclature and Diagnostic (INHAND) criteria for the cardiovascular system (13). Similarly, microscopic alterations in the lungs and kidneys were evaluated using modified INHAND criteria for the respiratory (14) and urinary system (15), respectively, with quantitative scoring. Liver and spleen histopathology were also assessed using scoring system based on a modified INHAND criteria for the hepatobiliary (16), and haematolymphoid systems (17). Detailed descriptions of all scoring systems for organ damage assessment based on INHAND are provided in Supplementary Table 1 (13,15–18).

3. RESULTS AND DISCUSSION

3.1. Acute Toxicity Assessment

Throughout the 14-day observation period, no mortality was recorded in any treatment group. All rats appeared clinically normal, showing no signs of toxicity such as lethargy, tremors, convulsions, or abnormal posture. A transient increase in respiratory rate was observed within the first 30 minutes post-gavage in rats receiving *Ciplukan* extract, likely attributable to procedural stress rather than toxic effects. No significant behavioral abnormalities were observed thereafter in any group. Behavioral observations are summarized in Table 2. Due to the absence of mortality or overt toxic effects at the highest tested dose (1200 mg/kg body weight), the LD_{50} of the *Ciplukan* extract could not be determined within the tested range.

As shown in Table 2, no mortality or overt signs of toxicity were observed at any dose level throughout the study. All monitored toxic signs, including convulsions, tremor, itching, coma, and mortality, were absent and marked as "NF" (Not Found), while behavioral parameters remained within normal limits. The transient increase in respiratory rate observed within the first 30 minutes post-administration was likely related to gavage-induced stress rather than a pharmacological response, as similarly reported in previous rodent studies (19).

3.2. Body Weight Changes

A consistent increase in body weight was observed across all groups, including the control group and treatment groups receiving different doses of *Ciplukan* extract (Table 3). The percentage increase in body weight remained below 20% relative to baseline measurements, indicating normal growth and the absence of treatment-related adverse effects. On day 14, a slight decline in body weight was observed in the G1 and G5 groups; however, these changes remained within the acceptable physiological range.

The absence of mortality and significant weight loss at doses up to 1200 mg/kg body weight suggests that the Indonesian *Ciplukan* extract possesses a relatively high safety margin for acute administration (Table 3). According to OECD guidelines, substances with LD_{50} values greater than 1000 mg/kg body weight are considered to have low acute oral toxicity (10). These findings provide essential toxicological data to support the regulatory and scientific basis for the safe development of *Ciplukan* as a standardized phytopharmaceutical, bridging traditional use with evidence-based clinical validation.

Table 1. Scoring system to evaluate organs damages based on INHAND classification

Organs	Score	Modified versions of the INHAND
Heart	0	Normal
	1	Inflammation/fibrosis/vacuolization is < 3 foci
	2	Inflammation/fibrosis/vacuolization is > 3 foci but not yet diffusely spread
	3	inflammation/fibrosis/vacuolization is diffusely spread
Lung	0	normal
	1	Alveolar wall thickening without lung structural damage
	2	Alveolar wall thickening/fibrosis accompanied by lung architectural damage
	3	Extensive fibrosis with honeycomb appearance
	4	Total lung damage
Kidney	0	No nuclear necrosis, tubular degeneration, or proximal tubular dilatation in any field of view
	1	Lesions such as nuclear necrosis, tubular degeneration, and proximal tubular dilatation found in several fields of view
	2	Lesions such as nuclear necrosis, tubular degeneration, and proximal tubular dilatation found in every field of view
Liver	0	Hepatocytes appear normal
	1	Hydropic degeneration, fatty degeneration, or necrosis focused in one location/field of view
	2	Hydropic degeneration, fatty degeneration, or necrosis focused in several locations/fields of view
	3	Hydropic degeneration, fatty degeneration, or necrosis present throughout all locations/fields of view
Spleen	0	Normal
	1	Focal haemorrhage/necrosis
	2	Multifocal haemorrhage/necrosis
	3	Diffuse haemorrhage/necrosis

Table 2. Behavioral observations from control and *Ciplukan* extract treatment groups

Parameters	Observation of Control and *Ciplukan* Extract Treatment Groups																			
	30 min					First 24 h (Intensive observation)					7 d					14 d				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Fur and skin	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	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	N	N	N	N	N	N	N	N
Respiration	N	↑	↑	↑	↑	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urine colour	N	N	N	N	N	N	N	N	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	N	N	N	N	N	N	N	N
Motor	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Activity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	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	N	N	N	N	N	N	N	N
Convulsions and tremor	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Itching	NF	NF	NF	NF	NF	NF	NF	NF	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	NF	NF	NF	NF	NF	NF	NF	NF
Mortality	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

Description: G1 = control group and received aqueous solution only; G2 = treated with *Ciplukan* extract at 100mg/kg BW; G3= treated with *Ciplukan* extract at 400mg/kg BW; G4 = treated with *Ciplukan* extract at 800mg/kg BW; G5 = treated with *Ciplukan* extract at 1200mg/kg BW; N = Normal; NF = Not Found

Table 3. Body weight (in grams) among control and *Ciplukan* extract treatment groups

Days	Body weight Median ± IQR from the research groups					p-value
	G1	G2	G3	G4	G5	
1st day	168.00 ± 15	169.00 ± 22	150.00 ± 25	154.00 ± 16	155.00 ± 11	0.226
7th day	191.00 ± 22	166.00 ± 29	174.00 ± 19	169.00 ± 13	165.00 ± 30	0.070
14th day	186.00 ± 29	168.00 ± 27	180.00 ± 21	179.00 ± 18	165.00 ± 29	0.269

Description: G1 = control group and received aqueous solution only; G2 = treated with *Ciplukan* extract at 100mg/kg BW; G3= treated with *Ciplukan* extract at 400mg/kg BW; G4 = treated with *Ciplukan* extract at 800mg/kg BW; G5 = treated with *Ciplukan* extract at 1200mg/kg BW

3.3. Food and Water Consumption

Daily food and water intake were comparable across all groups (Table 4). No statistically significant differences were observed between the control and treatment groups throughout the study ($p > 0.05$). These results indicate that administration of the *Ciplukan* extract did not affect appetite or hydration status, further supporting the extract's tolerability.

No mortality or overt signs of toxicity were observed at any dose level during the 14-day observation period (Table 2). All monitored toxic signs (convulsions, tremor, itching, coma, and mortality) were absent and recorded as "NF" (Not Found), while behavioral parameters remained within normal limits. A transient increase in respiratory rate was noted within the first 30 minutes after oral administration, likely attributable to gavage-related stress rather than a pharmacological effect, consistent with previous findings in rodent models (19).

3.4. Organ Weight

Organ weight assessment revealed no significant differences in the relative weights of the heart, lungs, kidneys, liver, or spleen between control and treated groups (Table 5). This suggests that acute administration of *Ciplukan* extract at the tested doses did not result in organ hypertrophy or atrophy.

3.5. Macroscopic Necropsy Findings

Macroscopic examination of the major organs revealed no visible abnormalities or macro pathological changes. The heart, lungs, kidneys, liver, and spleen appeared normal, with no observable discoloration, swelling, or lesions. The gross morphology of all organs was consistent across the control and treatment groups, indicating the absence of acute toxicity-related alterations following oral administration of the *Ciplukan* extract at the tested doses (Table 6).

3.6. Pathologic Findings

Histopathological analysis revealed dose-dependent microscopic alterations in multiple organs (Table 7 and Figure 1). The spleen exhibited the most pronounced changes, with median damage score increasing from 0.00 ± 0.00 in control group to 3.00 ± 1.00 in the high-dose group (1200 mg/kg). Moderate pathological changes were observed in the liver and heart at higher doses, whereas alterations in kidney and lung were generally milder. Kruskal-Wallis analysis confirmed statistically significant differences in in histopathological damage scores for the lungs ($p = 0.003$), kidneys ($p = 0.007$), liver ($p < 0.001$), and spleen ($p = 0.003$) among treatment groups, while the heart did not show a statistically significant difference ($p = 0.061$). Post-hoc Mann-Whitney analysis indicated that liver ($p = 0.003$) and spleen ($p = 0.005$) alterations became significant beginning at 400 mg/kg body weight. No significant histological changes were observed at 100 mg/kg compared to control, although early pathological features were evident in the spleen and kidneys.

Table 4. Daily food and water intake of female Sprague-Dawley rats during 14-day administration of *Ciplukan* extract

Group	Treatment	Food Intake (g/rat/day)			p-value	Water Intake (mL/rat/day)			p-value
		Day 1-5	Day 6-10	Day 11-14		Day 1-5	Day 6-10	Day 11-14	
G1	Control	15.3 ± 1.2	15.8 ± 0.9	16.1 ± 1.1		25.4 ± 2.3	26.2 ± 1.8	25.9 ± 2.1	
G2	*Ciplukan* extract 100 mg/kg BW	15.1 ± 1.3	15.5 ± 1.2	15.9 ± 1.0	0.872	24.9 ± 2.4	25.7 ± 2.1	25.6 ± 1.9	0.795
G3	*Ciplukan* extract 400 mg/kg BW	15.4 ± 0.8	15.7 ± 1.1	16.0 ± 0.9	0.914	25.6 ± 2.2	25.9 ± 2.0	25.8 ± 2.3	0.883
G4	*Ciplukan* extract 800 mg/kg BW	15.0 ± 1.1	15.4 ± 1.0	15.8 ± 1.2	0.764	25.1 ± 1.9	25.5 ± 2.2	25.4 ± 2.0	0.842
G5	*Ciplukan* extract 1200 mg/kg BW	14.8 ± 1.2	15.2 ± 1.3	15.7 ± 1.1	0.693	24.7 ± 2.1	25.3 ± 1.9	25.1 ± 2.2	0.719

Description: G1 = control group and received aqueous solution only; G2 = treated with *Ciplukan* extract at 100mg/kg BW; G3= treated with *Ciplukan* extract at 400mg/kg BW; G4 = treated with *Ciplukan* extract at 800mg/kg BW; G5 = treated with *Ciplukan* extract at 1200mg/kg BW

Representative histological images of organs from control and high-dose groups (1200 mg/kg) are presented in Figure 2. These images highlight vacuolization and mild fibrosis in cardiac tissue; alveolar wall thickening and interstitial fibrosis in the lungs; tubular dilation and epithelial degeneration in the kidneys; multifocal fatty degeneration and haemorrhage in the liver; and multifocal necrosis and haemorrhage in the spleen.

No significant differences in organ weights were observed between treatment and control groups, suggesting that acute administration of the extract did not induce major organ hypertrophy or atrophy. These findings align with previous studies on Golden Berry (*Physalis peruviana*) from Colombia, which reported body weight gain in treated animals but reduce organ and fat tissue weights after 16 days, along with improved glucose and lipid profiles (20). While earlier studies have suggested the general safety of *P. angulata* extracts at low doses (<5g/kg body weight) in mice without detailed organ assessment (21), the present work provides the first comprehensive acute toxicity evaluation following internationally standardized OECD protocols. By incorporating quantitative histopathological scoring of five vital organs, this study establishes a clear dose-response threshold of 400 mg/kg body weight for the Indonesian *Ciplukan* extract.

Table 5. Means of organ weight (in grams) among control and *Ciplukan* extract treatment groups

Organs	Groups					p-value
	G1	G2	G3	G4	G5	
Heart	0.77 ± 0.058	0.80 ± 0.140	0.82 ± 0.121	0.77 ± 0.084	0.69 ± 0.125	0.399
Right Lung	0.70 ± 0.143	0.86 ± 0.104	0.77 ± 0.118	0.78 ± 0.155	0.74 ± 0.122	0.499
Left Lung	0.57 ± 0.074	0.72 ± 0.167	0.74 ± 0.125	0.82 ± 0.202	0.70 ± 0.088	0.081
Right Kidney	0.64 ± 0.038	0.59 ± 0.039	0.60 ± 0.061	0.62 ± 0.050	0.58 ± 0.033	0.228
Left Kidney	0.64 ± 0.028	0.58 ± 0.058	0.57 ± 0.061	0.60 ± 0.064	0.54 ± 0.049	0.121
Liver	6.49 ± 0.331	5.87 ± 0.969	6.41 ± 1.103	6.70 ± 0.539	5.33 ± 0.537	0.051
Spleen	0.59 ± 0.053	0.47 ± 0.042	0.61 ± 0.176	0.58 ± 0.131	0.49 ± 0.121	0.179

Description: G1 = control group and received aqueous solution only; G2 = treated with *Ciplukan* extract at 100mg/kg BW; G3= treated with *Ciplukan* extract at 400mg/kg BW; G4 = treated with *Ciplukan* extract at 800mg/kg BW; G5 = treated with *Ciplukan* extract at 1200mg/kg BW

Table 6. Macroscopic necropsy findings from the organs of all control and experiment groups.

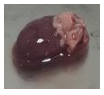
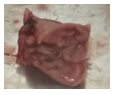
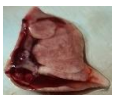
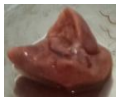
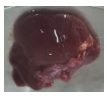
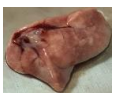
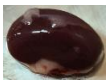
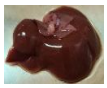
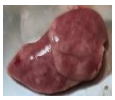
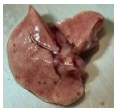
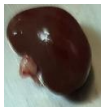
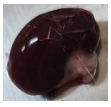
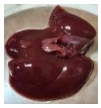
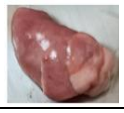
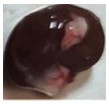
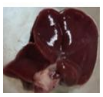
Group	Treatment	Heart	Right Lung	Left Lung	Right Kidney	Left Kidney	Liver	Spleen
G1	Control							
G2	Ciplukan extract 100mg/kg BW							
G3	Ciplukan extract 400mg/kg BW							
G4	Ciplukan extract 800mg/kg BW							
G5	Ciplukan extract 1200mg/kg BW							

Table 7. Scores of organ damages based of INHAND classification from control and treatment groups after *Ciplukan* extract exposures

Organs/Score	Median ± IQR				
	G1	G2	G3	G4	G5
Heart	0.00 ± 0.00	1.00 ± 1.00	2.00 ± 2.50	2.00 ± 3.50	2.00 ± 3.00
Lungs	0.00 ± 0.00	0.00 ± 0.50	1.00 ± 1.00	1.00 ± 0.00	1.00 ± 0.50
Kidneys	0.00 ± 0.00	1.00 ± 1.00	1.00 ± 1.00	1.00 ± 0.00	1.00 ± 0.00
Liver	0.00 ± 0.00	0.00 ± 1.00	2.00 ± 0.00	2.00 ± 0.50	2.00 ± 0.50
Spleen	0.00 ± 0.00	2.00 ± 0.00	2.00 ± 1.00	3.00 ± 1.00	3.00 ± 1.00

Description: G1 = control group and received aqueous solution only; G2 = treated with *Ciplukan* extract at 100mg/kg BW; G3= treated with *Ciplukan* extract at 400mg/kg BW; G4 = treated with *Ciplukan* extract at 800mg/kg BW; G5 = treated with *Ciplukan* extract at 1200mg/kg BW

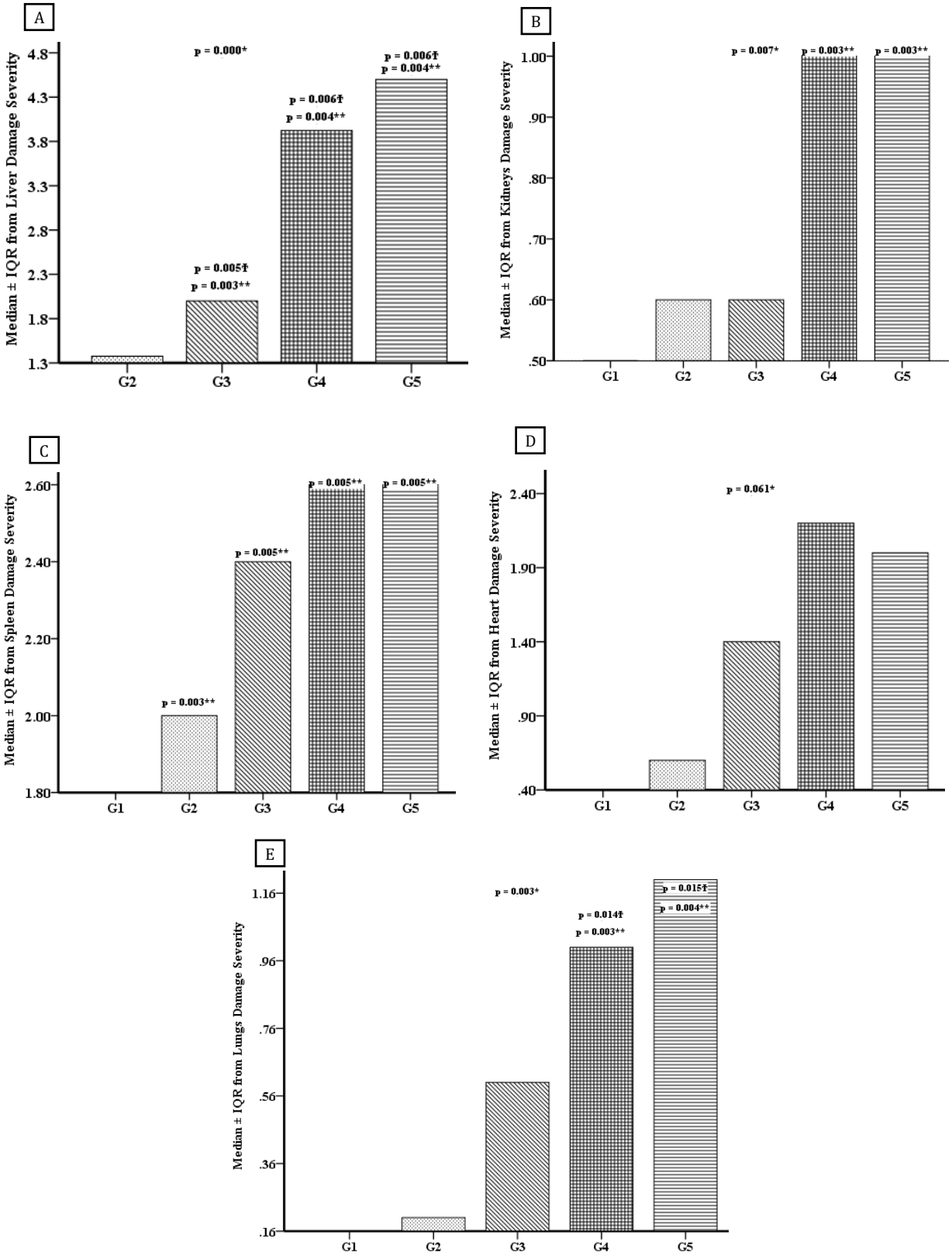


Figure 1. Comparison of Median ± IQR severity levels of organ damage in heart (A), lungs (B), kidneys (C), liver (D), and spleen (E). G1 = control group and received aqueous solution only; G2 = treated with *Ciplukan* extract at 100mg/kg BW; G3= treated with *Ciplukan* extract at 400mg/kg BW; G4 = treated with *Ciplukan* extract at 800mg/kg BW; G5 = treated with *Ciplukan* extract at 1200mg/kg BW. Description: * = Kruskal Wallis test; ** = Comparison to G1 group, Mann-Whitney test; † = Comparison to G2 group, Mann-Whitney test.

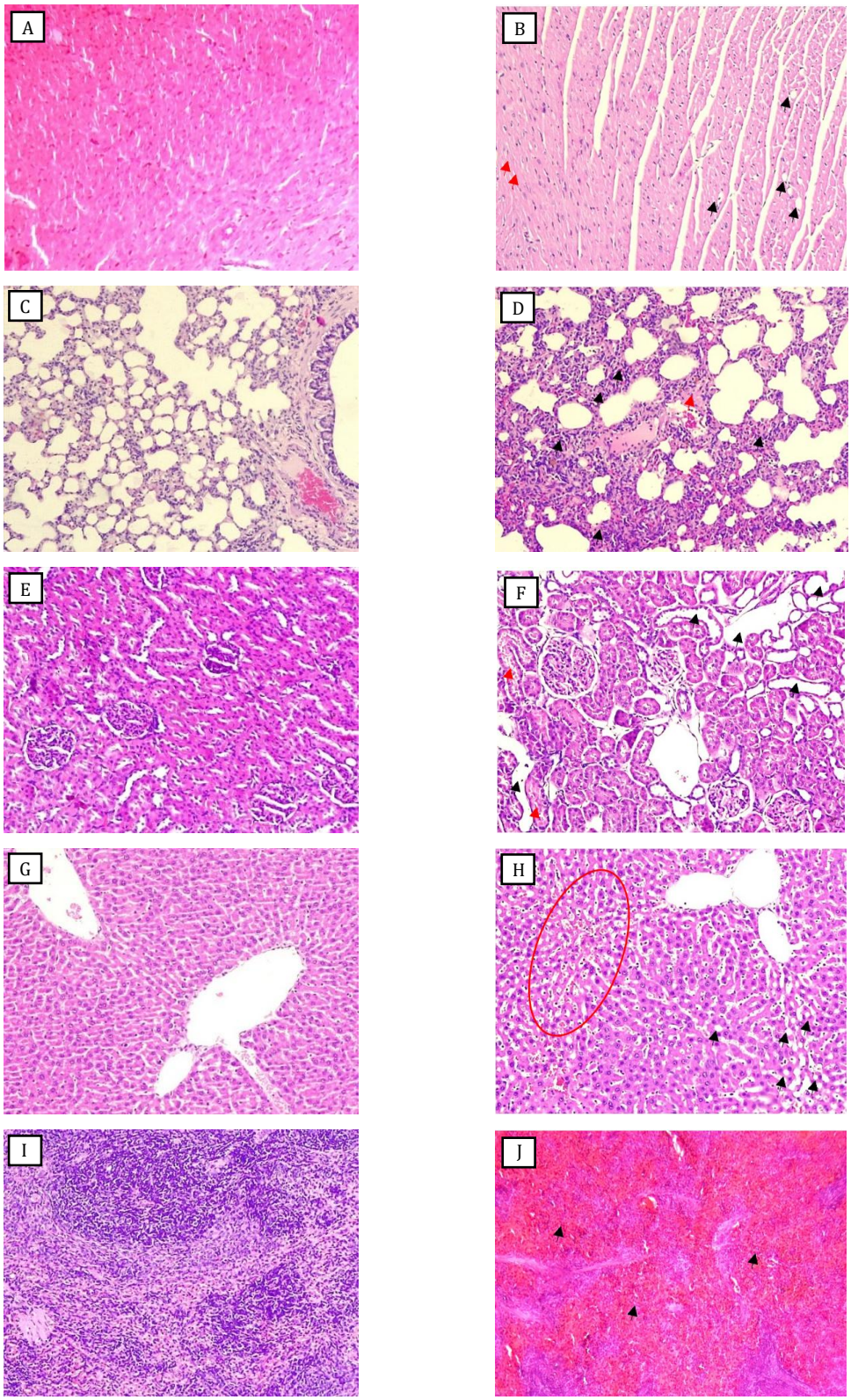


Figure 2. Microscopic changes in the investigated organs between control group (G1) and *Ciplukan* Extract 1200mg/kg BW group (G5). The heart appeared normal in group G1 (A), while in group G5, vacuolization (black arrow) and mild fibrosis (red arrow) (B) were observed; group G1 showed normal microscopic appearance of the lungs (C), but group G5 showed thickening of the alveolar wall (black arrow) and mild fibrosis (red arrow) without structural damage of the lungs (D); normal kidney appearance in group G1 (E), while tubular dilatation (black arrow) and tubular epithelial degeneration (red arrow) (F) were obtained from group G5 kidneys; normal liver structure was seen in group G1 (G), but in the liver structure of group G5, multifocal fatty degeneration (black arrow) and haemorrhage (red circle) were found (H); while in group G1 the splenic structure appeared normal (I), multifocal haemorrhage and necrosis of the spleen were found in group G5 (black arrow) (J). (H&E, 200x magnification, except 3c and 3d at 4x magnification). G1 = control group and received aqueous solution only; G5 = treated with *Ciplukan* extract at 1200mg/kg BW

Dose-dependent histopathological changes were particularly evident in the heart, lungs, kidneys, liver, and spleen at doses ≥400 mg/kg body weight. In cardiac tissue, vacuolization and early fibrotic features suggestive of collagen deposition were observed (Figure 2A-B), based on haematoxylin and eosin (H&E) staining showing increased extracellular matrix density and altered tissue architecture. Lung histopathology revealed alveolar wall thickening and early fibrotic at higher doses of the extract (Figure 2C-D). In the kidneys, significant alterations were observed at 800 and 1200 mg/kg, characterized by tubular dilation and epithelial degeneration (Figure 2E-F). Liver tissues exhibited multifocal fatty degeneration and haemorrhage beginning at 400 mg/kg, while spleen samples showed multifocal haemorrhage and necrosis starting at 100 mg/kg, although these early changes were not statistically significant compared to controls.

The observed dose-dependent organ alterations may be attributed to oxidative stress mediated by reactive oxygen species (ROS). *P. angulata* L. contains bioactive flavonoids, which, although known for their anti-inflammatory properties, can also induce oxidative stress at higher concentrations (22). Flavonoids modulate inflammation by suppressing key signaling pathways—such as Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Activator Protein-1 (AP-1)—that regulate pro-inflammatory cytokines including IL-1β, Tumour Necrosis Factor-α (TNF-α), and IL-6 (23). They also inhibit cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS), reduce the production of prostaglandins and nitric oxide, and modulate Mitogen-Activated Protein Kinase (MAPK), Phosphoinositide 3-Kinase Protein Kinase B (PI3K/Akt), and Toll-Like Receptor 4 (TLR4) signaling pathways, thereby attenuating neutrophil recruitment and overall inflammation (24).

In addition, flavonoids possess strong antioxidant properties, allowing them to neutralize free radicals that contribute to oxidative stress and inflammation. However, at higher doses, excessive ROS formation may exceed the antioxidant capacity of the system. However, at higher doses, excessive ROS formation may exceed the antioxidant capacity of the system. Under such conditions, antioxidants themselves can undergo oxidation, generating free radicals or failing to effectively scavenge ROS due to redox imbalance (25). This mechanism may explain the organ-specific damage observed in our study. In the heart, vacuolization and mild fibrosis were observed in treated groups, indicating early necrotic changes. This uncontrolled and irreversible cell death may form of cell death may result from calcium and ROS-induced damage to proteins, lipids, and DNA, ultimately compromising organelle and cell integrity (26,27). The loss of membrane integrity and subsequent intracellular fluid accumulation cause cell swelling and the formation of large cytoplasmic vacuoles, which is a hallmark of necrotic cell death or oncosis. This process arises from the leakage of ions such as sodium (Na^+) into the cell due to membrane dysfunction, followed by osmotic water influx, leading to cell swelling. The resulting organelle damage and eventual cell membrane rupture further aggravate inflammation and tissue injury (26,28). Persistent vacuolization is a marker of cellular injury and, when prolonged, promotes chronic inflammation that may lead to the replacement of normal parenchymal tissue with fibrotic connective tissue (29).

At higher doses, *Ciplukan* extract may increase the production of pro-inflammatory cytokines and Transforming Growth Factor-β1 (TGF-β1), promoting fibroblast activation, collagen synthesis, and extracellular matrix accumulation. These processes can contribute to alveolar wall thickening and mild fibrosis in the lungs, consistent with our histopathological findings (29). Furthermore, kidney tubular dilatation and epithelial degeneration were observed in groups receiving 800 and 1200 mg/kg body weight, significantly differing from the control group. These lesions may result from increased oxidative stress at higher *Ciplukan* extract doses, leading to ROS accumulation and subsequent cell membrane injury. Damage to tubular epithelial cells impairs reabsorption and secretion, resulting in tubular dilatation (30). In the liver, multifocal fatty degeneration and haemorrhage were observed across multiple microscopic fields. These alterations may occur because high doses of *Ciplukan* extract promote free radical accumulation and excessive hydrogen peroxide (H_2O_2) generation. Accumulated H_2O_2 may undergo Fenton reactions in the presence of intracellular ferrous iron (Fe^{2+}), producing highly reactive hydroxyl radicals (•OH). These radicals initiate lipid peroxidation of polyunsaturated fatty acids (PUFAs) in hepatocyte membrane phospholipids, leading to structural damage, reduced membrane fluidity, and pore formation. Such alterations disrupt the function of membrane proteins, including ion-transport enzymes such as Na^+/K^+-ATPase, ultimately disturbing ion homeostasis and promoting sodium and water influx into hepatocytes, resulting in cytoplasmic swelling and functional impairment (31). In addition, leakage of lysosomal hydrolytic enzymes may further contribute to cytoplasmic degeneration and macromolecular accumulation, leading to hydropic degeneration (32).

Excessive ROS attack cellular and organelle membranes through lipid peroxidation, producing malondialdehyde (MDA) and other peroxidation products, which further compromise membrane structure and function. In addition, flavonoids possess strong antioxidant properties, enabling them to neutralize free radicals that contribute to oxidative stress and inflammation. As a consequence of oxidative damage, hepatocytes may lose their normal metabolic function and begin accumulating lipids in response to cellular stress, resulting in fatty degeneration. Histopathological, fatty degeneration is characterized by cytoplasmic vacuolization, hepatocyte enlargement, cytoplasmic discoloration, lipid accumulation, and peripheral displacement of the nucleus (32). Hydropic degeneration represents an early stage of hepatocellular injury in which the structural integrity of the cell remains largely preserved, but metabolism and fluid homeostasis are impaired. Fatty degeneration constitutes the subsequent stage, in which continued membrane and organelle dysfunction further disrupts normal metabolic processes. If oxidative stress persists or damage becomes severe, hepatocytes lose their structural integrity and metabolic capacity, ultimately undergoing necrosis. Necrotic cells are

characterized by loss of nuclear structure (karyolysis, karyorrhexis, pyknotic) and amorphous, eosinophilic cytoplasmic damage, accompanied by enzyme release and surrounding tissue injury. Such necrosis can lead to more extensive inflammation and overall impairment of liver function (32). Multifocal splenic haemorrhages and necrosis were observed starting at 100 mg/kg *Ciplukan* extract. These pathological changes likely result from dose-dependent oxidative stress and ROS accumulation, leading to excessive nitric oxide production and endothelial injury (25). Furthermore, increased vascular permeability associated with disruption of endothelial junctions facilitates the leakage of fluid and blood into tissues, thereby elevating the risk of haemorrhage (33). Prolonged bleeding may cause tissue hypoxia and extensive cell death, culminating in necrotic lesion formation. While we propose that oxidative stress may underlie the observed organ damage based on the known bioactive compounds in *Ciplukan* and previously established mechanisms, oxidative stress markers were not directly measured in this study. Future investigations should include biochemical assessments of ROS levels, lipid peroxidation products, and antioxidant enzyme activities, such as superoxide dismutase, catalase, and glutathione peroxidase, to validate this mechanistic hypothesis.

Previous studies have shown that increasing doses of physalin, the major bioactive component of *P. angulata* L., can induce excessive autophagy, that triggers the activation of cell necrosis characterised by cell cytoplasmic vacuolization (34). The present study expands upon prior reports that primarily evaluated *P. angulata* extracts at lower doses or in short-term studies (21). In contrast, this research employs a systematic, OECD-guided toxicological framework, incorporated detailed histological grading across multiple organ systems. The identification of organ-specific damage at doses ≥400 mg/kg establishes a quantifiable safety threshold and provides valuable guidance for future dose selection in therapeutic development. These findings also align with traditional medicinal practices, which typically employ lower doses of this plant. Notably, the *Ciplukan* used in this study was sourced from Banyumas, Central Java—an area characterized by high humidity and intense sunlight, environmental factors that may influence secondary metabolite production Local communities traditionally use this plant to treat conditions such as arthritis, liver, malaria, and various skin diseases. Its long-standing use has shaped regional harvesting and preparation practices, which may in turn affect its chemical profile. Consequently, ecological and ethnomedical factors may render Indonesian *Ciplukan* distinct from varieties cultivated in other regions (35). This highlights the importance of region-specific toxicological validation, even for plants with well-documented traditional use.

Despite providing essential acute toxicity data, this study has several limitations: First, the absence of biochemical and molecular analyses, including oxidative stress biomarkers and antioxidant enzyme assays, limits the mechanistic interpretation of the observed histopathological changes. Second, the single-dose acute toxicity design restricts the ability to predict chronic or cumulative toxic effects, particularly given the dose-dependent tissue alterations observed at ≥400 mg/kg. Third, the absence of Masson's trichrome staining limits definitive confirmation of fibrosis; thus, future studies should incorporate collagen- specific staining to validate these findings. Finally, as this study was conducted exclusively in female rats, potential sex-related differences in toxicological response could not be assessed. Future research should include both sexes, a broader dose range, extended observation periods, and molecular profiling to develop a more comprehensive safety evaluation of Indonesian *Ciplukan* extract.

4. CONCLUSIONS

This study provides the first comprehensive evaluation of the acute oral toxicity of Indonesian *Ciplukan* (*Physalis angulata* L.) extract sourced from Mersi, Banyumas, Central Java, using female Sprague-Dawley rats. The findings indicate that the extract has an LD_{50} value exceeding 1200 mg/kg body weight, suggesting a favorable acute safety profile according to OECD Test Guideline 425. However, histopathological analyses revealed significant dose-dependent alterations in multiple organs—including the liver, spleen, kidneys, and lungs—beginning at doses of 400 mg/kg body weight. These results highlight the importance of dose optimization in the development of *Ciplukan*-based phytomedicines. Although no clinical signs of toxicity or mortality were observed, the microscopic alterations detected at higher doses suggests possible subclinical organ injury that could become more pronounced with repeated or prolonged exposure. Therefore, further sub-chronic and chronic toxicity studies are warranted to determine the no-observed-adverse-effect level (NOAEL), elucidate underlying mechanisms of toxicity, and assess biochemical and functional outcomes. These findings contribute to the scientific validation of *Ciplukan* as a potential medicinal plant and provide essential toxicological evidence to support its safe use in both traditional and modern therapeutic contexts.

Author contributions: TS, NSI: Conceptualized the study and contributed to the research design, Data interpretation, and Manuscript writing. S, DN: Participated in participant recruitment and preparation of the manuscript draft. TS, NSI, FA: Were involved in the analysis of clinical data. All authors critically reviewed the manuscript, approved the final version, and take full responsibility for the content and similarity index of the paper.

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Ethics statement: This study has been approved by the Medical Research Ethics Committee of FK UNSOED, Universitas Jenderal Soedirman, with approval number 039/KEPK/PE/VI/2022.

Conflict of interest: The authors declare that there are no conflicts of interest related to this article.

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