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RESEARCH ARTICLE

Standardisation and toxicity assessment of *Dioscorea hispida* (Dennst) tubers: Acute and subchronic dosage studies

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Abstract

People worldwide have used *Dioscorea hispida* Dennst to treat various skin diseases. However, *Dioscorea hispida* tubers are known to be poisonous because they contain cyanide and *dioscorine*. This research aimed to standardise *Dioscorea hispida* dried powder and examine tuber extract toxicity effects (acute and subchronic). This research was carried out with sample standardisation according to the Indonesian Herbal Pharmacopoeia II. The Regulations of the Republic of Indonesia Food and Drug Supervisory Agency carried out acute and subchronic toxicity studies. Wistar rats were administered different doses of the extract and observations were made for toxic symptoms, body weight changes, mortality, relative organ weights and histopathological changes in liver and kidney tissues. Serum creatinine, ALT and AST levels were also measured. *Dioscorea hispida* tubers from North Sumatra and Lampung met the quality standards, whereas those from West Java did not due to high mold/yeast counts. Acute toxicity studies indicated that doses of 300 mg/kg BW and 1000 mg/kg BW caused toxic symptoms and death, classifying the extract as toxic with an LD50 range of 50-500 mg/kg BW. No toxic symptoms were observed at 50 mg/kg BW. Subchronic studies revealed that elevated doses led to significant liver and kidney damage, evidenced by increased creatinine, ALT, AST levels and histopathological changes. *Dioscorea hispida* tubers from North Sumatra and Lampung meet quality standards, but those from West Java do not. The tubers have medicinal potential but exhibit high toxicity at elevated doses, necessitating cautious use and further research to ensure safe application and reduce contaminants.

Keywords

acute and subchronic toxicity; *Dioscorea hispida* tuber; extract; standardisation

Introduction

Dioscorea hispida Dennst is a significant biological resource in Indonesia with potential applications as an alternative medicinal plant and food source. Traditionally, various communities have utilised *D. hispida* to treat ailments such as leprosy (1), syphilis (1), calluses (2), vaginal discharge (3), menstrual pain (4), inflammation (5) and rheumatism (5). The medicinal properties of *D. hispida* are attributed to its rich content of secondary metabolites. However, *D. hispida* tubers are known to be toxic. The primary toxic component, *dioscorine*, is an alkaloid that can cause central nervous system paralysis. *D. hispida* tubers contain alkaloids, including free cyanide in hydrogen cyanide (HCN). The presence of these toxic compounds necessitates a comprehensive evaluation of the tuber's safety for medicinal use (5,6).

The safety of *D. hispida* extracts is determined through various toxicity tests, including acute, subchronic, chronic and specific toxicity assessments. Acute toxicity

tests detect immediate effects following a single or repeated dose within 24 h (7). These tests help determine the lethal dose (LD50), guide subsequent toxicity testing and classify the material's toxicity level. Subchronic toxicity tests, on the other hand, assess the effects of repeated exposure to the test substance over a more extended period, typically up to 10% of the test animal's lifespan. These tests provide insights into toxic effects not detected in acute tests, potential cumulative and reversible effects and safe dosage levels (8).

The use of dried powder, or crude drug materials, in traditional medicine is predominantly met through wild plant collection, with minimal cultivation. To ensure the quality and safety of dried powder, they must meet government-specified standards as outlined in the Indonesian Materia Medika or the Indonesian Herbal Pharmacopoeia (FHI) (9,10). Examination of mutual dried powder can be done organoleptically, macroscopically, microscopically, biologically and chemically. The organoleptic and macroscopic examination checks the purity of the quality of dried powder using the five senses that exist in humans (11). Organoleptic and macroscopic examinations can be carried out by observing external characteristics such as shape, colour, smell and taste. If an organoleptic examination has been carried out, it can be continued with a microscopic examination to observe the histological characteristics of the plant, primarily to ensure its authenticity. After conducting macroscopic and microscopic examinations, further examinations can be conducted to determine the quality based on the active compounds in the dried powder (9-12).

The use of *D. hispida* in traditional medicine has been well-documented, along with detoxification practices such as slicing, soaking, boiling and rubbing with ash, which aims to reduce cyanide content (13,14). Given its continued use in certain communities, ensuring the *in vivo* safety of *D. hispida* consumption is essential. However, to date, no studies have conducted both acute and subchronic toxicity testing specifically on the ethanol extract of *D. hispida* tubers. This research fills that gap by providing practical evidence of its safety and toxicity profile, supporting the development of guidelines for its safe medicinal use.

Based on the description above, it is imperative to ensure that *D. hispida* tuber dried powder meets the standard criteria set by the Indonesian Materia Medika or Indonesian Herbal Pharmacopoeia because these tubers are still obtained from the wild and have not been cultivated in the community. In addition, because its use in society is relatively high as an alternative treatment, it is necessary to assess the acute and subchronic toxicity profile to assess the harmful effects of its consumption and the influence of the compounds in *D. hispida* tubers.

Materials and Methods

Preparation of D. hispida tuber

The samples of *D. hispida* Dennst tuber were collected from three regions: Deli Serdang district, North Sumatra (212/MEDA/2022); Tenggamus district, Lampung (690/MEDA/2023); and Pangandaran district, West Java (687/MEDA/2023). Plant sample identification was conducted at the Medanense Herbarium Laboratory, Faculty of Mathematics and Natural Sciences, University of North Sumatra. The tubers (28.760 kg) were sliced thinly, dried at 50°C for 48 h in a hot air oven and ground into a fine powder yielding 4.5 kg of dried powder. A total of 4.5 kg of the dried powder was extracted with 97% ethanol (1:10 w/v) for 48 h. The extract was filtered and concentrated using a rotary evaporator, resulting in 160 g of viscous extract with a dried powder yield of 15.64% and an extract yield of 3.5% (10).

Standardisation of dried powder

D. hispida tuber dried powder was standardised according to the Indonesian Herbal Pharmacopoeia II. Dried powder standardisation testing was conducted on tubers from 3 regions: North Sumatra, Lampung and West Java Provinces. The tubers were tested for standardisation, including the assessment of specific and non-specific parameters. Specific parameter testing consists of organoleptic, microscopic examination, phytochemical screening, soluble essence content (water and ethanol), TLC chromatogram pattern, FTIR spectrum and total phenolic and flavonoid content. Meanwhile, non-specific parameters include total ash content, total acid soluble ash content, drying loss, water content, total plate number, mold/yeast number and heavy metal contamination (Pb and Cd) (9,10,15).

Acute oral dose toxicity study

The acute oral dose toxicity study was carried out per the Fixed Dose Method, outlined in Regulation Number 10 of 2022, issued by the Indonesian Food and Drug Authority. The study was divided into two phases: the starting and main phases. Assessments were performed on 4-week-old female Wistar rats (120-140 g). In the starting phase, six groups of three rats each were tested. The control group received 0.5% CMC-Na (w/v), while the treatment groups were given a single oral dose of the ethanol extract of *D. hispida* tubers suspended in 0.5% CMC-Na (w/v) at doses of 5, 50, 300, 1000 and 2000 mg/kg BW. The animals were observed closely for toxic symptoms and mortality during the first 4 h after administration and then monitored regularly for 14 days. The results from the starting phase guided the selection of doses for the main phase (7,8).

In the main phase, four groups of five rats each were tested, with each group including one rat from the starting phase and four newly introduced rats. The control group again received 0.5% CMC-Na (w/v), while the treatment groups were given doses of 50, 300 and 1000 mg/kg BW. The animals were observed following the same protocol as in the starting phase, with close monitoring during the first 4 h and at regular intervals for 14 days to assess delayed toxic effects and mortality (7,8).

Subchronic oral dose toxicity study

The subchronic oral dose toxicity study was carried out according to The Indonesian Food and Drug Authority Regulation No.10 of 2022, where the study is divided into the starting and main phases. Wistar rats (4 weeks old; 120-140 g) were divided into six treatment groups, with each group comprising 10 rats (5 males and 5 females). The treatment groups were: Group I (control) received 0.5% CMC-Na; Groups II, III and IV received doses of 75, 150 and 300 mg/kg BW, respectively, suspended in 0.5% CMC-Na. Group V (satellite control) received 0.5% CMC-Na and Group VI (toxic satellite) received a dose of 300 mg/kg BW. Each dose was administered daily for 14 days and all animals were closely monitored throughout this period. Satellite groups were further observed for an additional seven days to assess any delayed toxic effects (7,8).

Parameters analysed for toxicity study

Toxic symptoms and clinical observations

Toxic symptoms and clinical observations were conducted on test animals to assess both acute and sub-chronic oral toxicity. For acute oral toxicity, observations focused on monitoring toxic symptoms, body weight and mortality, with symptoms such as changes in skin colour, eye colour, shaking, tremor, diarrhoea, lethargy, backward walking and stomach walking noted for 2 h post-administration and monitored daily for 14 days. In the sub-chronic oral toxicity study, test animals were observed over 14 days, with an additional seven days for satellite groups, evaluating parameters including toxic symptoms, body weight and mortality. Toxic symptoms and clinical signs observed were similar to those in the acute toxicity study, including changes in seizure, weakness, changes in feathers, changes in the ocular mucosa, backward walking and stomach walking (7,8).

Relative organ weight measurement

Relative organ weight measurements were carried out by measuring the absolute weights of the liver and kidney. The average relative organ weight was calculated as the organ weight divided by the body weight immediately before sacrifice. These calculations allow for the normalization of organ weights to account for variations in animal body size, providing a standardized measure of organ size relative to body weight, expressed in mg/100 g BW (16,17).

Macroscopic and histopathological examination

The collected organs, including the liver and kidney, underwent thorough visual inspection to assess macroscopic changes, focusing on colour, surface texture and consistency to identify signs of toxicity or pathological alterations such as discolouration, surface irregularities and altered tissue consistency. The organs were fixed in 10% formalin for histopathological examination to preserve tissue structure and then processed using standard paraffin embedding techniques. The embedded tissues were sectioned into thin slices and stained with hematoxylin and eosin to highlight cellular details and structural features. These stained sections were examined under a microscope at 10x magnification to evaluate tissue morphology and identify pathological changes. This detailed histopathological analysis, conducted exclusively for the acute and sub-chronic toxicity study, provided insights into the long-term cellular and tissue-level impacts of the ethanol extract of *D. hispida* tubers (18,19).

Liver and kidney function assessments

The hearts of sacrificed animals were subjected to centrifugation at 3000 rpm for 15 min after 0.5 ml of blood was extracted. The serum was obtained and dispatched to the laboratory to evaluate creatinine, ALT and AST levels. This was just conducted for the subchronic study (20,21).

Statistical analysis

The numerical values of the control group and different treated groups were analysed for their differences using *One-Way Analysis of Variance* (ANOVA) followed by a post-hoc test of Tukey HSD using statistical analysis software Statistic Product and Service Solutions (SPSS). One-way ANOVA (Tukey HSD) and probability value were considered significant when $p<0.05$. All values were expressed as mean ± SD (7).

Results and Discussion

The dried powder tubers of *D. hispida* Dennst were studied in three different regencies: Pangandaran (West Java), Tenggamus (Lampung) and Deli Serdang (North Sumatra). This selection represents Indonesia's diverse geography and biodiversity. Pangandaran is 0-1 km above sea level and receives an average annual rainfall of 2.5 m. Deli Serdang is 0 to 0.5 km above sea level and its annual rainfall averages range from 0.13 to 0.29 m. Tenggamus is located at 0 to 2 km altitude and its annual rainfall averages 0.17 m. These variations in altitude and rainfall are anticipated to affect the tubers' chemical composition and qualitative features.

Standardisation with specific parameters

Specific parameter testing included organoleptic properties, microscopic examination, phytochemical screening, soluble essence (water and ethanol), TLC chromatogram pattern, FTIR spectrum, total phenolic content and flavonoid content (9,10,12). Organoleptic observations of *D. hispida* tubers revealed variations across regions: tubers from North Sumatra were dark brown, odourless and tasteless with a rough, irregular surface, measuring 9-20 cm in length and 8-13 cm in width. Tubers from Lampung were 9-12 cm long and 6-13 cm wide, while those from West Java were 10-11 cm long and 9-13 cm wide. The prepared *dried powder* had a yellowish-white color, rough surface, distinctive smell and tastelessness, with slices ranging from 2-4 cm in length and 1-2 cm in width. Microscopic examination confirmed the presence of concentric-type starch, raphide-shaped calcium oxalate crystals, parenchyma cells, transport bundles with ladder-like thickening and fibres, ensuring consistency across samples from all three regions (see Tables 1 & 2).

The phytochemical screening of *D. hispida* dried powder and extracts from North Sumatra, Lampung and West Java confirmed the presence of secondary metabolites, including flavonoids, saponins, alkaloids, glycosides, tannins and steroids/terpenoids, with no differences observed between regions (Table 3) (11). Additionally, the tubers from all regions met the requirements for water-soluble and ethanol-soluble essence content, with higher concentrations of water-soluble compounds. Statistical analysis showed no significant differences in soluble essence content among the regions, suggesting that environmental factors such as soil nutrients, climate, altitude and growing conditions likely influenced the compound composition (Table 4) (22,23).

TLC chromatogram pattern testing of *D. hispida* tuber dried powder from North Sumatra, Lampung and West Java was conducted by observing the spots under visible light, short-wave ultraviolet (254 nm) and long-wave ultraviolet (366 nm) before and after spraying with $FeCl_3$. The Rf values across regions showed minimal differences, indicating consistent secondary metabolite content (Tables 5 & 6). Environmental factors, such as soil nutrients, climate and altitude, likely influenced the minor variations observed. Testing with $FeCl_3$ revealed blue spots at Rf 1 and Rf 4 and brownish spots at Rf 7 and Rf 8, confirming the presence of phenolic compounds in samples from all three regions (24).

The Fourier Transform Infrared (FTIR) investigation results in Fig. 1 show that the absorption wavenumbers detected at 3456, 2931, 1639 and 1233 cm^{-1} correspond to the strain frequencies of

Table 1. Organoleptic observations

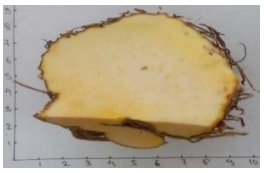
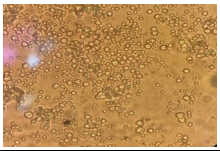
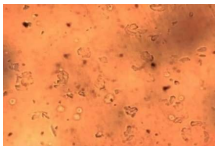
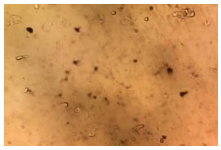
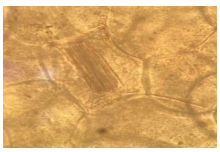
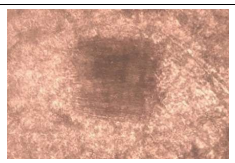
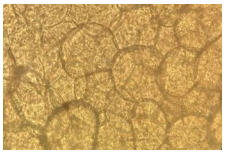
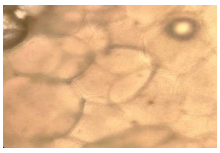
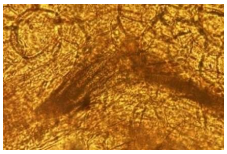
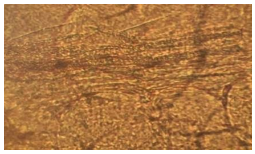
Tubers of *Dioscorea hispida* from North Sumatra	Tubers of *Dioscorea hispida* from Lampung	Tubers of *Dioscorea hispida* from West Java
		
		
		
		

Table 2. Microscopic observations

Tubers of *Dioscorea hispida* from North Sumatra	Tubers of *Dioscorea hispida* from Lampung	Tubers of *Dioscorea hispida* from West Java
Starch with concentric type (10 × 100)		
		
Ca Oxalate Crystals Rafid crystal form (10 × 40)		
		
Parenchyma (10 × 40)		
		
Carrier network with ladder thickening (10 × 40)		
		
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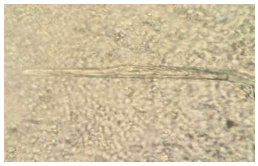
		

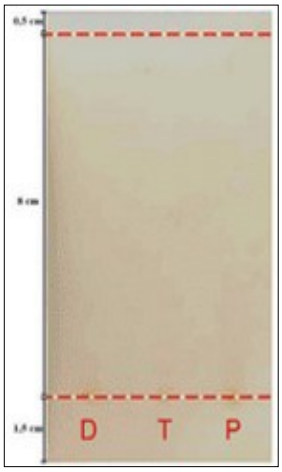
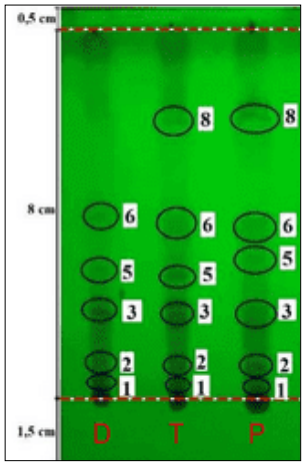
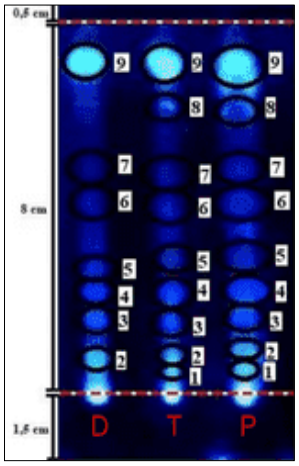
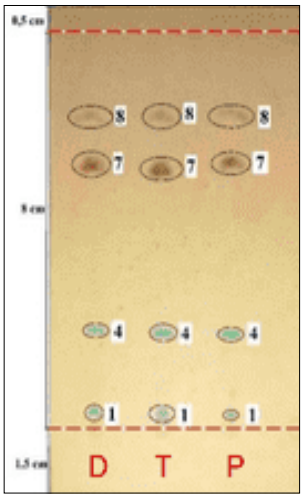
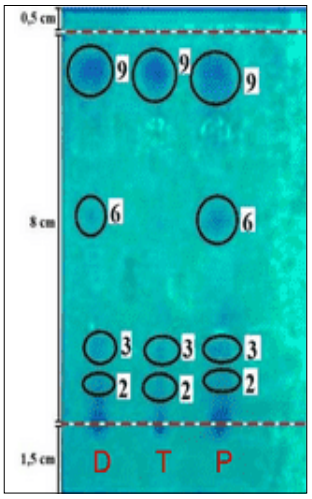
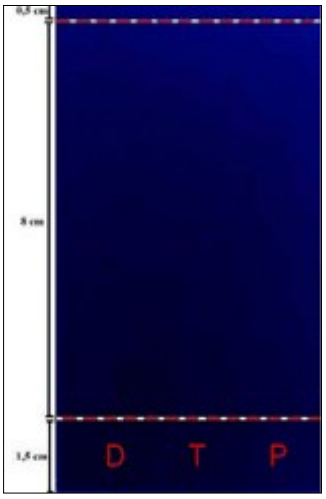
Table 3. Phytochemical screening

No.	Compound Classes	Dried powder	Extract
1	Flavonoids	+	+
2	Saponin	+	+
3	Alkaloids	+	+
4	Glycosides	+	+
5	Tannin	+	+
6	Steroids / Triterpenoids	+	+

Table 4. Essence content in water and ethanol

No.	Parameter	Tubers of *Dioscorea hispida* from			Requirements
		North Sumatra	Lampung	West Java	
1.	Water Soluble Essence Content	10.10%	6.06%	12.43%	≥ 6%
2.	Ethanol Soluble Essence Content	4.05%	4.12%	4.46%	≥ 4%

Table 5. TLC chromatogram pattern

Observation	Direct Observation	UV 254	UV 366
Before spraying with FeCl3 spotting agent			
After spraying with FeCl3, spotting			

Description: D: North Sumatra T: Lampung P: West Java

Table 6. Rf Value of TLC chromatogram pattern

Spotting	Tubers of *Dioscorea hispida* from		
	North Sumatra	Lampung	West Java
Rf 1	0.0375	0.0375	0.0375
Rf 2	0.0875	0.1	0.1125
Rf 3	0.1875	0.1875	0.1875
Rf 4	0.25	0.2375	0.2375
Rf 5	0.3375	0.3625	0.3625
Rf 6	0.5	0.5	0.5
Rf 7	0.6625	0.65	0.6625
Rf 8	0.7875	0.7875	0.8
Rf 9	0.8875	0.8875	0.8875

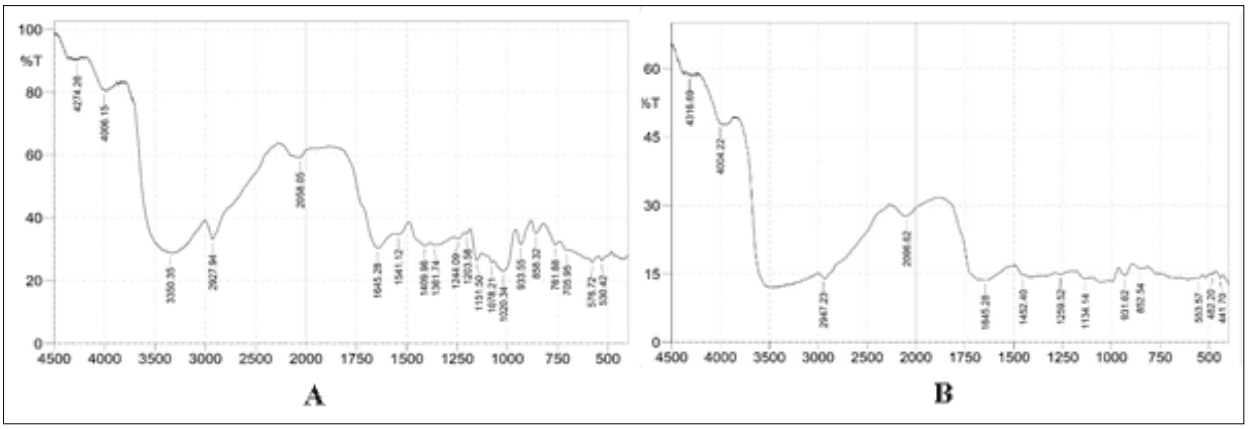


Fig. 1. FTIR Spectra of Dried powder (A) and Extract (B) of *Dioscorea hispida* (Dennst.) Tubers.

different functional groups. The wave number 3456 cm^{-1} indicates the -OH functional group and the wave number 2931 cm^{-1} indicates the -C-H functional group. The wave number at 1639 cm^{-1} shows the C-O (carbonyl) functional group, while the wave number at 1233 cm^{-1} shows the -C-O-C functional group. Identifying this functional group indicates the possible presence of polyphenolic, flavonoid and alkaloid compounds in the *D. hispida* (Dennst.) tubers extract (25).

The Folin-Ciocalteau reagent was used to quantify total phenolic content by forming a blue complex with phenolics under alkaline conditions, while the aluminium chloride colorimetric method measured total flavonoid content through complexation with aluminium chloride (11). As shown in Table 7, the total phenolic content of *D. hispida* tuber dried powder from North Sumatra, Lampung and West Java was 47.76%, 47.16% and 50.19%, respectively, with gallic acid equivalents determined using the regression equation y = 0.0014x + 0.0029. Flavonoid content was 0.34%, 0.31% and 0.27%, respectively, based on the equation y = 0.0073x + 0.0017. Statistical analysis revealed significant regional differences in phenolic and flavonoid levels, likely influenced by environmental factors such as soil nutrients, climate, altitude, temperature and CO_2 levels, all of which can enhance secondary metabolite production (11,22).

Standardisation with non-specific parameters

The non-specific parameter testing of *D. hispida* dried powder included total ash content, total acid-soluble ash, drying loss, water content, microbial contamination (total plate count, mold/yeast) and heavy metal levels (Pb and Cd) (Table 8). The total ash content, indicating inorganic residue, was 2.29% for North Sumatra, 1.33% for Lampung and 1.58% for West Java, all within the acceptable limit of <6%. Higher ash content reflects lower sample quality due to the presence of inorganic matter. Significant differences in ash content were observed between North Sumatra and Lampung but not between Lampung and West Java, likely influenced by regional environmental conditions (9,10,12).

The acid-insoluble ash content, which identifies inorganic compounds like soil or sand, was 0.45%, 0.24% and 0.55% for North Sumatra, Lampung and West Java, respectively, all below the 1% limit. Drying loss, measured after 30 min at 105°C, ranged from 8.24% in North Sumatra to 6.50% in West Java. Water content analysis using the azeotropic method showed values of 7.99%, 5.32% and 7.93% across the three regions, meeting the required standards. Microbial and fungal counts, based on total plate numbers and mold/yeast testing, were within acceptable limits, ensuring sample safety (9,10,12). Heavy metal testing via atomic absorption spectroscopy confirmed that Pb and Cd levels were below regulatory limits, indicating no heavy metal toxicity risks. Environmental factors such as soil nutrients, climate, altitude and CO_2 levels likely contributed to the variations in quality and safety of the *D. hispida* tuber dried powder (26,27).

Acute toxicity results

Toxic symptoms and clinical observations

Based on the initial acute toxicity test observations (Table 9), which adhere to the Indonesian Food and Drug Supervisory Agency Regulation Number 10 of 2022, the main test was conducted at 50, 300 and 1000 mg/kg body weight. This decision was made because the test animals administered doses of 1000 and 2000 mg/kg body weight died within 14 days of *D. hispida* extract administration. During the main acute toxicity test (Table 10), the animals exhibited limpness and convulsive behaviour, attributed to the toxic effects of dioscorin found in *D. hispida* tubers. Dioscorin interacts with nicotinic acetylcholine receptors

Table 7. Total level of phenols and flavonoids

Parameter	Tubers of *Dioscorea hispida* from		
	North Sumatra	Lampung	West Java
Total Phenol	47.76%	47.16%	50.19%.
Total Flavonoids	0.34%	0.31%.	0.27%.

Table 8. Non-Specific parameters of *Dioscorea hispida* tuber dried powder

No.	Parameter	Tubers of *Dioscorea hispida* from			Requirements
		North Sumatra	Lampung	West Java	
1	Total Ash Content	2.29%	1.33%	1.58%	≤ 6%
2	Acid Insoluble Ash Content	0.45%	0.24%	0.55%	≤ 1%
3	Drying loss	8.24%	7.55%	6.50%	-
4	Water content	7.99%	5.32%	7.93%	< 10%
5	Total Plate Numbers	1510 colony/g	370 colony/g	530 colony/g	<10000 colony/g
6	Mold/Yeast Numbers	510 colony/g	25 colony/g	7300 colony/g	<1000 colony/g
7	Pb Metal Contamination	0.1171 µg/ml	0.1801 µg/ml	0.1351 µg/ml	< 10 µg/ml
8	Cd Metal Contamination	0.0390 µg/ml	0.0372 µg/ml	0.0450 µg/ml	< 0.3 µg/ml

Table 9. Observation result of acute toxicity testing starting phase

Parameters	Normal control	Dose 5 mg/kg	Dose 50 mg/kg	Dose 300 mg/kg	Dose 1000 mg/kg	Dose 2000 mg/kg
Average BW (g)	134.67 ± 3.51	133.0 ± 10.44	129.67 ± 6.65	129.0 ± 6.55	131.0 ± 7.937	127.0 ± 4.58
Average BW (after two w) (g)	149.33 ± 14.18	161.33 ± 12.50	162.0 ± 10.0	165.33 ± 7.57	163.0 ± 0	-
Skin color	Normal	Normal	Normal	Normal	Normal	Normal
Eye color	Normal	Normal	Normal	Normal	Normal	Normal
Shaking	-	-	-	-	-	-
Tremor	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-
Lethargy	-	-	-	+	+	+
Backwards walking	-	-	-	-	-	-
Stomach walking	-	-	-	-	-	-
Mortality	0	0	0	0	2	3

n = 3; values are expressed as mean ± SD

Table 10. Observation result of acute toxicity testing main phase

Parameters	Normal control	Dose 50 mg/kg	Dose 300 mg/kg	Dose 1000 mg/kg
Average BW (g)	128.00 ± 8.27	129.20 ± 5.97	130.00 ± 6.63	130 ± 6.11
Average BW (after two w) (g)	144.00 ± 13.24	133.0 ± 9.72	159.67 ± 2.51	-
Skin color	Normal	Normal	Normal	Normal
Eye color	Normal	Normal	Normal	Normal
Shaking	-	-	-	-
Tremor	-	-	+	+
Diarrhea	-	-	-	-
Lethargy	-	-	+	+
Backwards walking	-	-	-	-
Stomach walking	-	-	-	-
Mortality	0	0	2	5

n = 5; values are expressed as mean ± SD

(nAChR), causing local anaesthetic effects; a 0.5% dioscorin solution shows almost the same activity as a 0.05% cocaine solution. Although dioscorin also exhibits antidiuretic and depressant activity, large doses can cause convulsions, with death usually resulting from extensor spasms (7,8).

Relative organ weights

Observations of the relative weights of liver and kidney organs in the main acute toxicity test revealed no significant differences between the control group and the various dose groups. This suggests that administering *D. hispida* tuber ethanol extract does not significantly affect organ weights. While behavioural changes were evident at higher doses, there was no substantial impact on organ weights, indicating that the extract did not cause significant organ toxicity at the tested doses (7,8). Based on Table 11, statistical analysis of the relative organ weights for the liver and kidney using *One-Way Analysis of Variance* (ANOVA), followed by Tukey's HSD post hoc test, revealed no significant differences between the experimental groups.

Macroscopic and histopathological observations

Macropathological examination of liver and kidney tissues assessed organ color, surface texture and consistency to identify

Table 11. Average relative organ weight index of liver and kidney

Groups	Average organ weight (mg/100 g BW)	
	Liver	Kidney
Normal control	27.08 ± 2.78	2.99 ± 0.35
Dose 50 mg/kg	30.08 ± 5.26	3.46 ± 0.53
Dose 300 mg/kg	23.90 ± 3.14	2.39 ± 0.19
Dose 1000 mg/kg	23.27 ± 1.26	2.64 ± 0.13

n = 5; values are expressed as mean ± SD

toxic effects and target organ damage. As shown in Fig. 2, the liver of all test animals appeared brownish-red with a smooth surface and firm consistency, while the kidneys exhibited a similar brownish-red hue with sleek texture and resilience. Microscopic observations at 10× magnification revealed that the control group had normal liver tissue with occasional black spot necrosis of hepatocytes. In contrast, mice administered 50 mg/kg BW of *D. hispida* ethanol extract showed pink tissue hemorrhage and those given 300 mg/kg BW exhibited sinusoidal dilation due to hepatocyte contraction, with extensive hepatocyte necrosis observed (Table 12) (18,19). Liver damage included parenchymatous degeneration, characterized by cloudy cytoplasm and mild cellular swelling, alongside progressive forms

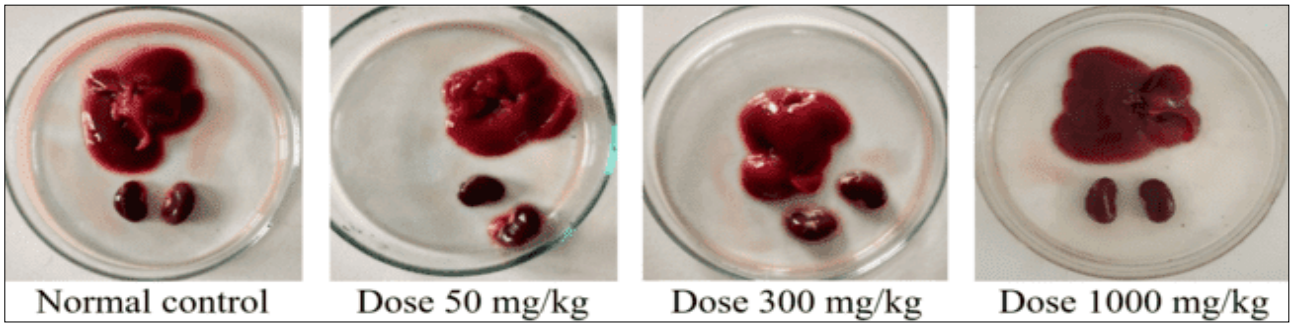
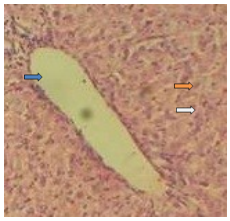
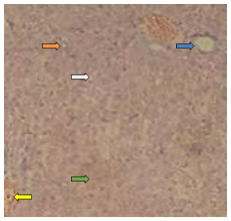
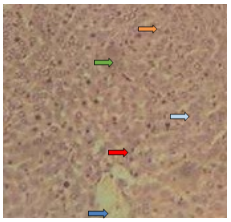
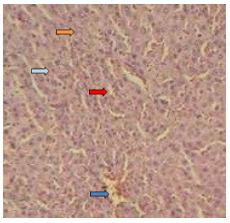
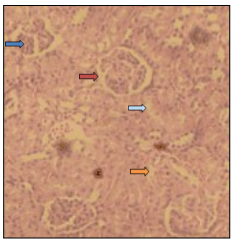
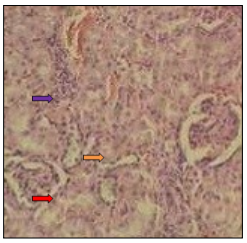
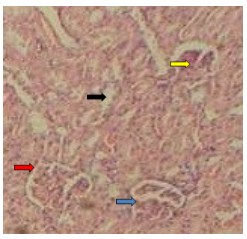
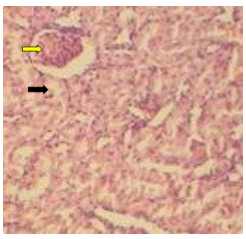


Fig. 2. Macroscopic observation of liver and kidney in acute toxicity testing.

Table 12. Results of histological observations of the liver and kidney in acute toxicity

Liver histology			
			
Normal control	**Dose 50 mg/kg**	**Dose 300 mg/kg**	**Dose 1000 mg/kg**
Description: → Central vein; → Hepatocytes;→ Sinusoid; →Bleeding; → Dilation of sinusoids; → Parenchymatous degeneration; → Cell necrosis			
Kidney histology			
			
Normal control	**Dose 50 mg/kg**	**Dose 300 mg/kg**	**Dose 1000 mg/kg**
Description: →Glomerulus; →Bowman's Capsule; →Proximal Tubule; →Distal Tubule; →Bowman space narrowing;→ Basophilic tubules; →Tubular Dilatation;→ Glomerular Atrophy			

of necrosis, such as pyknotic nuclei (compact, dark-stained nuclei), karyokinesis (nuclear fragmentation) and karyolysis (disappearance of the nucleus). Hydropic degeneration, involving water accumulation in the cytoplasm, was also noted as a more severe cellular response (18,19).

Kidney examination showed no toxic effects in the control group, while reversible damage was observed in the 50 mg/kg BW dosage group, including narrowing of Bowman's space and the appearance of basophilic tubules. In the higher dosage groups (300 mg/kg BW and 1000 mg/kg BW), more severe damage occurred, including further narrowing of Bowman's space, tubular dilation and glomerular atrophy. Toxic insult to the tubular epithelium can disrupt absorption and excretion, leading to tubular dilation; however, mild dilation may occur as a normal histological variant. Hepatocyte necrosis was also observed in control animals, likely due to natural cell regeneration for tissue homeostasis. While no liver necrosis was seen in the 50 mg/kg BW group, significant necrosis and loss of portal vein epithelium occurred at 300 mg/kg BW, indicating the hazardous effect of the extract at higher doses. Similarly, while reversible damage such as Bowman's space constriction was noted at 50 mg/kg BW, irreversible damage, including glomerular atrophy, was evident at higher doses, suggesting that recovery from toxic effects may not be possible at elevated exposure levels (18,19).

Subchronic toxicity results

Toxic symptoms and clinical observations

The ethanol extract of *D. hispida* was administered to male and female rats at doses of 75, 150 and 300 mg/kg body weight (BB) to investigate the harmful effects of the extract. The study was completed for 14 days, plus an extra seven days specifically for the satellite group. Toxic symptoms, body mass index, death rate, relative organ weights, macropathology, histopathology of kidney and liver tissues and creatinine, ALT and AST values were among the parameters assessed. Symptoms of toxicity, including convulsions, weakness, fur changes, ocular mucosa abnormalities, reverse walking and stomach walking, were observed daily for 14 days and 2 h after injection. Furthermore, the mortality and body weight of the animals were documented (7,8). The detailed observations of these parameters are summarised in Table 13.

Table 13. Observation results of subchronic toxicity testing

Parameters	Normal control		Dose 75 mg/kg		Dose 150 mg/kg		Dose 300 mg/kg		Normal control (Satelite)		Dose 300 mg/kg (Satelite)	
	M	F	M	F	M	F	M	F	M	F	M	F
Average BW (g)	129,2 ±14,5	133,0 ±13,8	128,6 ±15,8	125,4 ±18,6	114,8 ±9,4	137,6 ±7,0	130,2 ±18,1	115,4 ±15,2	123,2 ±9,4	134,4 ±7,5	126,4 ±8,6	128,6 ±11,8
Average BW (after two w) (g)	139,6 ± 8,6	134,8 ± 17,2	149,2± 8,3	131,6 ±18,9	120,5 ±14,5	129,8 ±22,3	148,0 ±0	147,0 ±0	123,6 ±12,7	140,4 ±9,0	134 ±0	143 ±0
seizure	-	-	-	-	-	-	+	+	-	-	+	+
weak	-	-	-	-	-	-	+	+	-	-	+	+
changes in feathers	-	-	-	-	-	-	+	+	-	-	+	+
changes in the ocular mucosa	-	-	-	-	-	-	+	+	-	-	+	+
Backwards walking	-	-	-	-	-	-	-	-	-	-	-	-
Stomach walking	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	0	0	1	0	1	0	4	4	0	0	4	4

n = 5; values are expressed as mean ± SD

Observations revealed no substantial alteration in body weight among the control, test and satellite groups. Animals used in experiments can have changes in their body weight caused by both internal and external factors. Internal factors include genetics and the control of hormones. External factors include diet, sunlight, activity level, temperature and environmental conditions. The 300 mg/kg BW dose group and the 300 mg/kg BW satellite group exhibited the highest mortality rate, with 80% of the test animals succumbing. Toxic levels can impair physiological activities, especially in essential organs and body chemistry, resulting in death, making mortality the most severe outcome of toxic effects from an extract. The ethanol extract of *D. hispida* tubers contain poisonous alkaloid dioscorin, with an LD50 of 0.09 g/kg BW (28).

Relative organ weights

The relative organ weight index of the liver was influenced by the administration of ethanol extract of *D. hispida* at varying oral concentrations, as shown in Table 14. The liver was chosen for analysis because it processes, detoxifies, stores and excretes xenobiotics and their metabolites, rendering it vulnerable to toxic harm. Organ weight is a sensitive indicator of liver impact, with abnormalities characterised by increased size and weight and enlargement and thickening of the liver lobules. When exposed to hazardous substances, the liver must work harder to compensate, which can increase the liver's weight (16,17).

Due to the kidney's sensitivity to toxicants, the kidney-organ ratio was also evaluated for subchronic toxicity. The kidney-organ ratio of the test animals given the extract rose compared to the group that served as the control, mainly when the doses were raised. This rise in size could be attributed to the presence of inflammation, which leads to an expansion of the kidney. The antihypertensive impact of Dioscorin can lower blood pressure and decrease blood flow to the kidney, leading to hypoxia in the kidney tubular epithelial cells. Extended and severe lack of oxygen can cause cell death, marked by more giant cells due to inflammation, leading to an enlarged kidney (16,17).

Macroscopic and histopathological observations

Macropathological examination showed that the liver and kidneys appeared normal in Fig. 3, with a smooth surface, springy consistency and brownish-red coloration. A healthy liver typically displays no changes in color, structure, or texture, as variations can indicate toxicity or organ damage. As summarized in Table 15, parenchymal degeneration was observed in male and female rats given *D. hispida* ethanol extract at 75, 150 and 300 mg/kg BW, as well as in the satellite group receiving 300 mg/kg BW. This degeneration involved hepatocyte swelling with cloudy, granular cytoplasm due to protein accumulation. Hydropic degeneration was also found in the 300 mg/kg BW group and the satellite group, characterized by pale, water-filled cytoplasm devoid of fat. Both parenchymal and hydropic degeneration are reversible, though hydropic degeneration reflects more severe cellular damage by interfering with ion transport, particularly sodium (Na^+) exchange across cell membranes (19,29).

Hepatocyte necrosis was identified in rats treated with *D. hispida* ethanol extract at 75, 150 and 300 mg/kg BW, as well as in the satellite group. Necrosis progresses through three stages-pyknosis (shrinkage and darkening of the nucleus), karyorrhexis (nuclear fragmentation) and karyolysis (loss of the nucleus). At

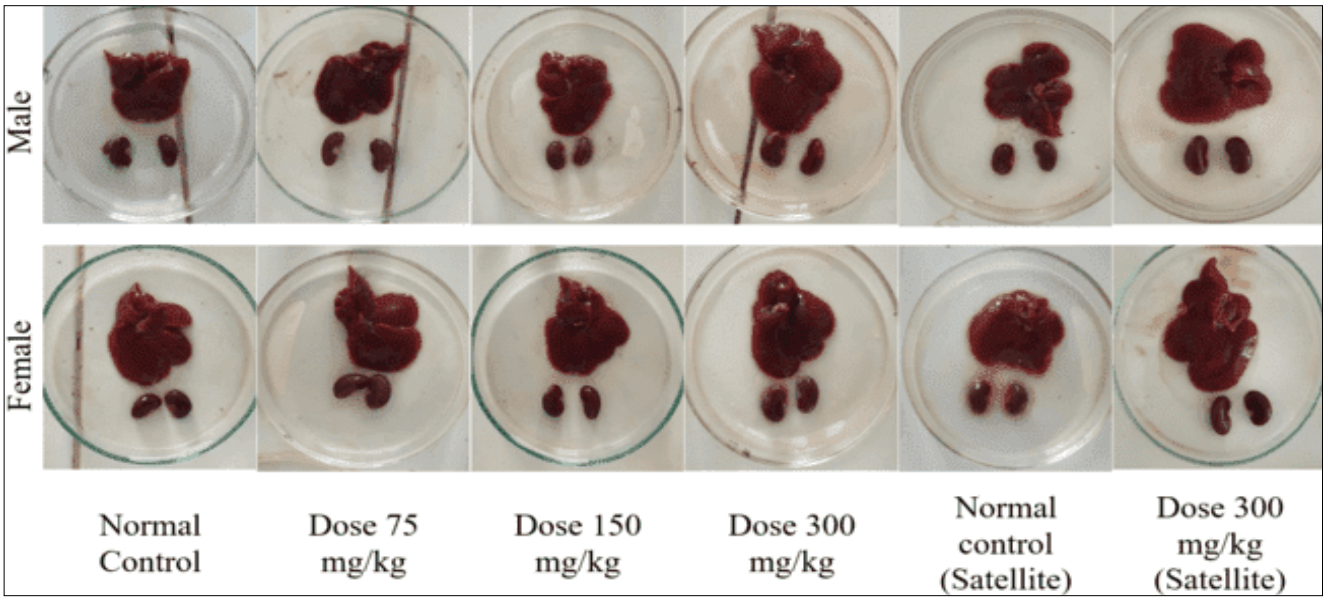


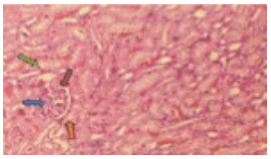
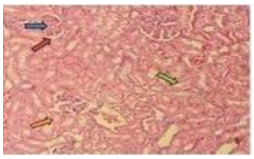
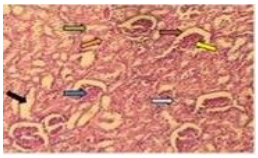
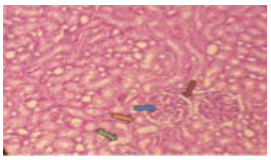
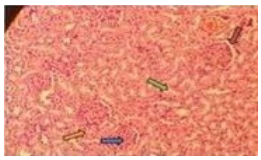
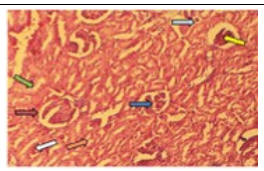
Fig. 3. Macroscopic observation of the liver and kidney in subchronic toxicity study.

Table 14. Average relative organ weight index of liver and kidney

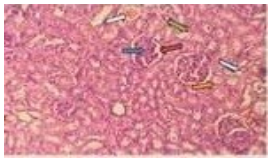
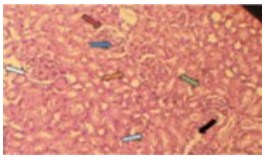
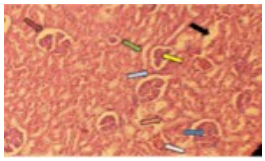
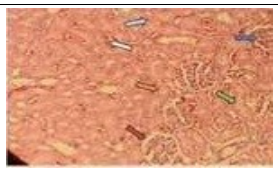
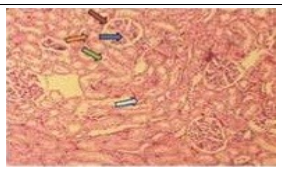
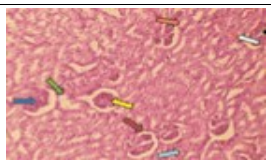
Groups	Average organ weight (mg/100 g BW)			
	Liver		Kidney	
	M	F	M	F
Normal control	35.4 ± 0.8	35.2 ± 2.9	3.8 ± 0.2	3.9 ± 0.2
Dose 75 mg/kg	34.4 ± 2.6	35.9 ± 3.0	4.3 ± 0.1	4.0 ± 0.2
Dose 150 mg/kg	41.7 ± 5.4	33.8 ± 2.2	4.3 ± 0.1	4.0 ± 0.1
Dose 300 mg/kg	37.0 ± 5.7	50.2 ± 5.8	4.8 ± 0.2	5.0 ± 0.2
Normal control (Satelite)	35.7 ± 3.2	33.4 ± 2.8	4.3 ± 0.3	3.8 ± 0.2
Dose 300 mg/kg (Satelite)	39.4 ± 3.9	47.5 ± 2.5	4.7 ± 0.1	4.5 ± 0.2

n = 5; values are expressed as mean ± SD

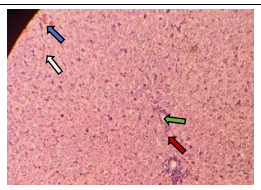
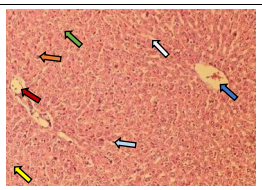
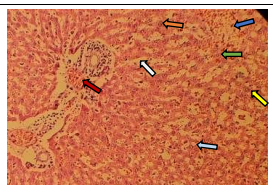
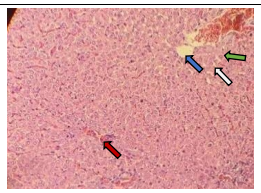
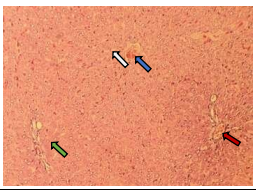
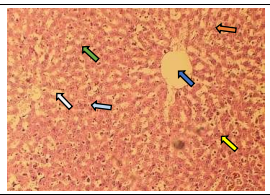
Table 15. Results of histological observations of the liver and kidney in subchronic toxicity study

Kidney histology			
Group	**Normal Control**	**Normal Control Satelite**	**Dose 300 mg/kg (Satelite)**
Male			
Female			

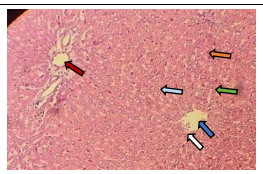
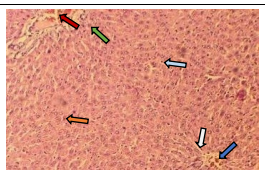
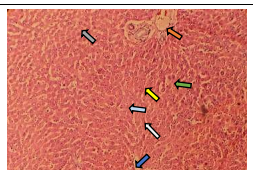
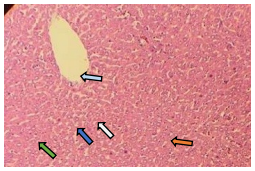
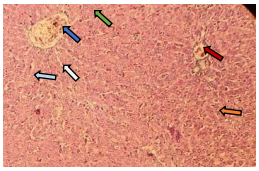
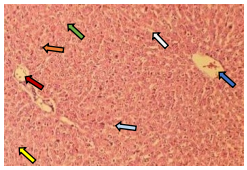
Description: → glomerulus →: Bowman's capsule →: proximal tubule →: distal tubule: necrosis →: bleed mark →: atrophy on glomerulus and →: dilation on tubule.

Group	**Dose 75 mg/kg**	**Dose 150 mg/kg**	**Dose 300 mg/kg**
Male			
Female			

Description: → glomerulus →: Bowman's capsule →: proximal tubule →: distal tubule: necrosis →: bleed mark →: atrophy on glomerulus and →: dilation on tubule.

Liver histology			
Group	**Normal Control**	**Normal Control Satelite**	**Dose 300 mg/kg (Satelite)**
Male			
Female			

Description: → central vein→: porta vein →: necrosis →: hepatocyte →: parenkimatic degeneration →: sinusoid and →: hydropic degeneration.

Group	**Dose 75 mg/kg**	**Dose 150 mg/kg**	**Dose 300 mg/kg**
Male			
Female			

Description: → central vein→: porta vein →: necrosis →: hepatocyte →: parenkimatic degeneration →: sinusoid and →: hydropic degeneration.

300 mg/kg BW, sinusoidal dilation was also observed, likely caused by disrupted lobular organization due to necrotic hepatocytes (20,30). Additionally, hemorrhage was detected across groups, presenting as petechiae, ecchymoses and streaky paint-brush patterns, suggesting vascular injury. Glomerular atrophy occurred in the kidneys of animals receiving 300 mg/kg BW extract and the satellite group, likely caused by increased Bowman's space pressure from altered glomerular permeability. Tubular dilation, seen at 150 and 300 mg/kg BW, reflects toxic epithelial injury that impairs renal absorption and secretion, though mild dilation may be considered a normal histological variation (31-34).

Liver and kidney function assessments

The normal creatinine values are 0.5-0.8 mg/dl. The results show that both male and female rats were given different amounts of *D. hispida* ethanol extract, with the satellite group experiencing abnormally high creatinine levels when given 300 mg/kg BW. High amounts of creatinine mean that the kidneys are not working as well as they should, primarily because of free radicals. Free radicals, unstable compounds with unpaired electrons, aggressively stabilise. Stress during the experiment, like being overcrowded, having uneven food, or having behaviour problems, can make more free radicals. Cell death occurs when cell contents bind to fibronectin in the tubular lumen, blocking creatinine excretion (20,21).

The liver has a crucial function in metabolic processes and an indication of harm to liver cells is increased levels of liver enzymes in the blood, notably aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Typically, AST and ALT levels are modest because of their intracellular nature under normal circumstances. Nevertheless, tissue injury leads to the breakdown of cells, releasing these enzymes into the bloodstream and causing an elevation in their concentration in the serum. The typical range of alanine aminotransferase (ALT) values in rats is between 34.9 and 218.1 U/L. Table 16 shows that male and female rats given *D. hispida* ethanol extract at 150 and 300 mg/kg BW had elevated ALT levels and the satellite group at 300 mg/kg BW exceeded normal limits. Liver injury can lead to the release of ALT and AST enzymes from hepatocytes into the bloodstream, causing an increase in serum levels that exceed the normal range (20,21).

Conclusion

The quality standardization of *D. hispida* tuber dried powder from North Sumatra and Lampung met regulatory requirements, while the samples from West Java exceeded acceptable fungal and yeast contamination limits. Toxicity testing of the ethanol extract revealed toxic symptoms and mortality at doses of 300 mg/kg BW and 1000 mg/kg BW, with no toxic effects observed at 50 mg/kg BW, placing the LD50 in the range of 50-500 mg/kg BW. Although some toxic effects were reversible after discontinuation, irreversible damage to the liver and kidneys was evidenced by elevated levels of creatinine, ALT and AST. These findings indicate the need for precise dosage control to minimize toxic effects while utilizing *D. hispida* extract.

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Authors' contributions

M conceived the study, participated in its design and coordination and funded the study. HS performed the standardization and toxicity analyses and contributed to the drafting and editing of the manuscript. AC performed the studies and analyses for acute toxicity. SFL performed the studies and analyses for acute toxicity. MWBS performed specific and nonspecific standardization studies. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT, an AI-based language model, solely to assist in structuring sentences and improving the clarity of language. After using this tool, the author(s) thoroughly reviewed and edited the content as needed and take full responsibility for the content of the publication.

Table 16. Creatinine, ALT and AST concentration of different groups in subchronic toxicity study

Groups		Creatinine conc. (mg/dl)	ALT conc. (U/L)	AST conc. (U/L)
Normal control	**Males**	0.5840 ± 0.049	154.80 ± 24.884	152.40 ± 14.519
	Females	0.5880 ± 0.044	131.80 ± 24.904	176.00 ± 10.747
Dose 75 mg/kg	**Males**	0.6550 ± 0.064	195.50 ± 17.711*	184.00 ± 21.643*
	Females	0.6480 ± 0.071	192.80 ± 20.753*	194.40 ± 14.519*
Dose 150 mg/kg	**Males**	0.8450 ± 0.062*	244.50 ± 39.753*	191.50 ± 12.477*
	Females	0.8180 ± 0.164*	230.60 ± 24.815*	212.20 ± 17.564*
Dose 300 mg/kg	**Males**	1.0400 ± 0*	278.00 ± 0*	312.00 ± 0*
	Females	0.9300 ± 0*	281.00 ± 0*	366.00 ± 0*
Normal control (Satelite)	**Males**	0.6640 ± 0.061	157.60 ± 25.618	164.20 ± 23.188
	Females	0.6380 ± 0.115	143.20 ± 21.833	176.80 ± 22.264
Dose 300 mg/kg (Satelite)	**Males**	0.9800 ± 0*	309.00 ± 0*	304.00 ± 0*
	Females	0.8800 ± 0*	318.00 ± 0*	315.00 ± 0*

n = 5; values are expressed as mean ± SD

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