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Research Article

Subacute Toxicity Test of Hydrocotyle Sibthorpioides Lam. Extract on *Histopathological Images* of Liver and Kidney of White Male Mice

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ABSTRACT

Introduction: *Hydrocotyle sibthorpioides* Lam. in Indonesia known as pegagan embun. It has been used to increase the immune system and has been shown to have immunostimulating, anti-inflammatory and hematopoietic effects. However, there is no scientific evidence that shows this plant is safe for long-term use. Based on that circumstance, this study aimed to measure the safety of Pegagan Embun (*Hydrocotyle sibthorpioides* Lam.) ethanol extract activities on liver and kidney histopathology. **Aim:** The study aimed to measure the safety of Pegagan Embun (*Hydrocotyle sibthorpioides* Lam.) ethanol extract activities on liver and kidney histopathology. **Material and Method:** Ethanol extract used because all the active compounds in plants extracted as a whole, and it cheaper and more efficient in the extraction process. Determine as many thirty-six white male mice as test animals and separate them into eight treatment groups. The administrated ethanol extract of Pegagan Embun (*Hydrocotyle sibthorpioides* Lam.) at doses of 7, 35, and 150 mg/kg BW for 7, 14, and 21 days. On days 8th, 15th, and 22nd, three white male mice collected from each treatment group and collected their liver and kidney. The data analysed used a T-test with IBM SPSS type 24. **Result:** LD50 of ethanol extract of *Hydrocotyle sibthorpioides* Lam. > 15,000 mg/kg means practically not toxic. The results showed that the administration of extract *Hydrocotyle sibthorpioides* Lam. for 7, 14, and 21 days showed a non-significant effect on any histological damage to the liver of male white mice at doses of 7 and 35 mg/kg BW (normal histology). The non-significant effect also occurs at150 mg/kg BW for 7 days; however, it caused mild damage at a dose of 150 mg/kg BW for 14 days and moderate damage at 150 mg/kg BW for 21 days. In renal histopathology, doses of 7 mg/kg BW. for 7, 14, and 21 days showed normal histology and doses of 35 mg/kg BW for 7, 14, and 21 days showed minimal damage. The administration at doses of 150 mg/kg BW for 7 days showed mild damage, while a dose of 150 mg/kg BW for 14 and 21 days showed moderate damage. **Conclusion:** It concluded that the administration of extract of *Hydrocotyle sibthorpioides* Lam. did not cause severe damage to the histology of the liver and kidneys of white male mice.

Key words: *Hydrocotyle sibthorpioides* Lam., Histopathology, Kidney, LD_{50}, Liver, Subacute.

INTRODUCTION

Indonesia has many beneficial plants for medicine, and it used for generations.[1] According to "*Badan Pengawas Obat dan Makanan"* (BPOM), or the National Agency for Drug and Food Control of Indonesia, there are 1.845 plant species identified as medicinal plants, 283 of which have officially registered as drugs. At the same time, the rest are still used traditionally.[2] Over the last two years, traditional medicines have increased globally. Research done by Lam *et al.* (2021) showed an increase in the use of traditional medicines from 48.4% to 54.1% during the COVID-19 pandemic.[3] Due to the pandemic, traditional medicine has been widely used to increase the immune system.[4]

One of the plants that can increase the immune system is *Hydrocotyle sibthorpioides* Lam. It is a traditional Chinese medicine that used to boost immunity.[5] Previous studies reported that the ethanolic extract of *Hydrocotyle sibthorpioides* Lam. was an immunostimulant. It showed by increasing the phagocytes' activity and capacity of macrophage cells.[6] In addition, it also reported that the ethanol extract of *Hydrocotyle sibthorpioides* Lam. has a significant effect on increasing leukocytes and the percentage of leukocytes in lymphocytes and neutrophil segments in male mice using the carbon clearance method.[7] Ethanolic extract of *H. sibthorpioides* also has a significant result as an anti-inflammatory. It showed that topical administration of the gel preparation of *H. sibthorpioides* ethanol extract reduces the volume of exudate in male white mice.[8]

Another research by Yu *et al.*[9] stated that the ethanolic extract of *Hydrocotyle sibthorpioides* has effects on mice's immunological function and produces an excellent antitumor effect. Wahyuni[10] reported that the ethanolic extract of *Hydrocotyle sibthorpioides* increased the activity of NK cells and CD8 cells in male white mice exposed to virus antigens. Husni *et al.*[11] reported that the ethanol extract of *Hydrocotyle sibthorpioides* increased the number of erythrocytes, reticulocytes, haemoglobin level, and hematocrit value in anaemic white male mice.

A toxicity activities test defined as a test conducted to examine the toxic effect of the substance on the biological system to acquire dose-response data. The acute toxicity test is one of the toxicity tests used to discover hazardous effects that occur quickly. Acute toxicity tests performed to assess a substance's fatal dose (LD50) and recommend dosage for extended studies.[12]

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LD50 defines as single doses of the substance which statistically hoped to kill 50% of the animal test. LD50 assessment was the first step in the toxicity test. It conducted by counting on animal test mortality in the first twenty-four hours after a single dose of substance administration until the fourteenth day.[13]

According to the Republic of Indonesia Ministry of Health,[1] the requirements for traditional medicine must be safe, beneficial, and of good quality. BPOM RI[2] stated that the tests determining traditional medicines' quality and safety standards include acute, subacute, chronic, subchronic, and specific toxicity tests. Toxic effects can occur because of the bond between the toxicant and liver cells.[14] After being absorbed, toxic substances and medicinal substances enter the blood circulation, detoxify the liver into harmless substances and then excrete them through the kidneys.[15] Therefore, it is crucial to evaluate the toxic effect on these two organs.

The data on *Hydrocotyle sibthorpioides* Lam. as herbal medicine safety has not obtained yet. For this reason, it examines the *Hydrocotyle sibthorpioides* Lam. could used as a standardised herbal medicine by determining the LD_{50} and conducting toxicity test to show its effect on liver and kidney tissue damage, which are the main organs in the body's metabolism and excretion. Thus, this research conducted to complement the safety data of *Hydrocotyle sibthorpioides* to develop into a standardised herbal medicine. Based on the explanation above, this study aims to observe the histology of the liver and kidneys after administering ethanol extract *Hydrocotyle sibthorpioides* Lam. The liver is an organ that is susceptible to the effects of toxic substances.

MATERIALS AND METHODS

Tools

The tools used were analytical scales (Ohaus), animal scales (Mip-lo), animal cages, measuring cups (Iwaki), mortar and pestle, dropper pipette, spatula, sonde (Terumo), beaker glass (Pyrex), ointment pot, tweezers (Lai Gooi TS), surgical scissors, object glass (Onelab), cover glass (Onelab), incubator (Memmert In110), freezer (Sharp SJ-246GC-SD), laminar air flow (Sugold Sw-Cj-1c), water bath (Memmert Wnb 22), staining jar, dehydration device (Tissue-Tek VIP 5 Jr), embedding center (Microm), embedding cassette (Macrosette), stopwatch, rotary microtome (Leica), computer, and microscope (olympus).

Materials

Hydrocotyle sibthorpioides Lam., aqua dest (Andeska laboratory), physiological NaCl (PT. Widatra Bhakti), Hematoxylin Ehrlich and eosin staining (Pupick Med), formalin buffer (Leica), alcohol 80% (Brata Med), alcohol 95 % (Brata Med), absolute ethanol (Brata Med), xylol 80% (Merck), xylol 95% (Merck), xylol 100% (Merck), Mayer's albumin, Canadian balm (Merck), paraffin (Surgipath).

Preparation of extraction

As much as 650g of *Hydrocotyle sibthorpioides* Lam. leaf dried, finely ground, and macerated using 70% ethanol as a solvent. Put a part of dry simplicia powder into a container, then add ten parts of solvent. Soak for the first 6 hours while stirring occasionally, then let stand for 18 hours and filter using filter paper. Macerate would obtain as a result of the filtering. The filtering process was repeated two times with the same type and amount. Collect all the macerate, then evaporate with a rotary evaporator until a thick extract obtained.

Extract standardisation

The extract standardised to non-specific and specific parameters. Non-specific parameters include drying shrinkage, total ash content, and acid insoluble ash content. While the specific parameters carried out include organoleptic, chemical content test, thin-layer chromatography, and determination of total flavonoid content.

Flavonoid

The 50 mg extract dissolved in 1-2 mL of ethanol in a test tube. Add 100 mg of mg metal or magnesium P powder and ten drops of HCl P. The presence of flavones and aurons and a red-orange to red-purple colour shows the presence of flavonoids in the extract.[16]

Treatment of test animals

The experimental study conducted to determine the effect of the administration of ethanol extract *Hydrocotyle sibthorpioides* Lam. at several dose levels by observing the liver and kidneys of white male mice histologically. As many as thirty-six white male mice aged ±2-3 months weighing 200-250g then divided into four treatment groups (three test groups and one control group). The test group given ethanol extract of *Hydrocotyle sibthorpioides* Lam. doses of 7, 35, and 150 mg/kg BW, while the control group given 0.5% Na CMC. Tests carried out on the 7^{th}, 14^{th}, and 21^{st} days. On days 8^{th}, 15^{th}, and 22^{nd} day three mice taken from each group, and then the liver and kidney taken for histopathological examination.

Ethical clearance

All animal test in this study has been getting ethical approval from The Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, with contract number 401/UN.16.2/KEP-FK/2021 for toxicity test on histopathological kidney and liver with contract number 404/UN.16.2/KEP-FK/2021 for LD_{50} measurement.

Toxicity test

Three parameters observed in this study are toxic symptoms, the percentage change in body weight, and animal mortality. Test animals were given preparations according to a single dose per oral—dose level. In the control group, only 0.5% Na CMC was given. Preparations are given based on the weight of the animal. Observations were made intensely on the first day for 4 hours, then periodically for 14 days.[17] Food given after 1-2 hours of administering the test preparation.

Animals that are still alive observed for toxic symptoms that arise. The animal's weight monitored from before offering the preparation until the animal sacrificed. After 14 days, they sacrificed by way of anaesthesia.

Histopathological

Histopathological preparations were prepared and observed using a microscope with a magnification of 400x. The data analysed quantitatively based on liver and kidney histopathological scores shown in Tables 1 and 2.

Steatosis, also called fatty change, occurs on hypoxic injury and various toxic or metabolic injuries.[19] Inflammation or inflammatory cells are the reactions of living tissue to all forms of injury in the form of a vascular response which results in the delivery of fluid, dissolved substances, and cells from the blood circulation to the interstitial tissue in the injured area.[20] Necrosis is a condition of injury to cells that results in the premature death of living cells and tissues. Necrosis occurs due to too much material reabsorbed by hepatocyte cells, which makes cell death.[21] Fibrosis is tissue in or around the portal tract or central vein due to severe fatty liver accompanied by inflammation or even liver cell death.[19]

Data analysis

The data obtained from body weight changes percentages then statistically analysed with one-way ANOVA. Then the significant result

Table 1: Liver histopathology score.[18]

Damage Type	Damage Rate	Percentage	Score
Steatosis	Normal	<10%	0
	Light	10-30%	1
	Moderate	31-60%	2
	Heavy	>60%	3
Inflammation	Normal	<10%	0
	Light	10-30%	1
	Moderate	31-60%	2
	Heavy	>60%	3
Necrosis	Normal	<10%	0
	Light	10-30%	1
	Moderate	31-60%	2
	Heavy	>60%	3
Fibrosis	Normal	<10%	0
	Light	10-30%	1
	Moderate	31-60%	2
	Heavy	>60%	3

Table 2: Renal histopathology score.[22]

Damage Percentage	Damage rate	Score
Normal Histology	Normal	0
<10% degeneration	Minimum	1
<25% degeneration	Light	2
<50% degeneration	Moderate	3
<75% degeneration	Heavy	4

analysed with Duncan Multiple Range Test (DMRT) with IBM SPSS 24 Version. Animal test mortality obtained and then analysed with the LD_{50} measurement formula based on Indonesian Herbal Pharmacopoeia Third Edition.

RESULTS

In testing the non-specific parameters of the extract, the drying shrinkage was 8.91%, the total ash content was 8.82%, and the acid insoluble ash content was 2.3%. While testing the specific parameters, the organoleptic results of the ethanol extract of *Hydrocotyle sibthorpioides* Lam. were in the form of a dark brown viscous liquid that tasted bitter and had a specific odour. Phytochemical testing results showed that the ethanolic extract of *Hydrocotyle sibthorpioides* Lam. contains phenolic secondary metabolites, flavonoids, saponins, and terpenoids.

The chromatogram pattern tested with thin layer chromatography using routine comparison compounds. The mobile phase used is an n-butanol mixture: acetic acid: water (BAA) in a ratio of 4:1:5, while the stationary phase is silica gel F254.[23] The results on the TLC plate with UV-Visible 254 nm light produced the same Rf value of the sample and comparison compound, as much as 0.6 (Figure 1).

The next test is the determination of total flavonoid levels. A routine comparison compound with a concentration series of 100, 75, 50 and 25 ppm.[19] Absorption measurements carried out at a maximum wavelength of 415 nm. The results obtained were ethanol extract of *Hydrocotyle sibthorpioides* Lam. containing flavonoid content of 1.34%.

The administration of *Hydrocotyle sibthorpioides* Lam. ethanol extract in the control group, treatment at doses of 1, 2, 4, and 8 g/kg BW did not show any toxic symptoms. The animal treatments moved actively and showed normal behaviour after administering the test preparation. Toxic symptoms found at a dose of 16 g/kg BW and 32 g/kg BW showed a little weak movement (moves slowly) and decreased motor activity. However, these symptoms only lasted for the first 1-2 hours after administering the test preparation, after which the mice were active again. Toxic symptoms increased at a dose of 64 g/kg BW; mice became weak, slightly rapid breathing, slight piloerection, and walking with the stomach slightly bent down. There was a decrease in motor activity in mice, indicated by the slow movement of the mice even though they were touched. At a 128 g/kg BW dose, more signs of toxicity. Animal treatments fragile, diarrhoea with very soft stool consistency, slight tremor, walking with the stomach bent down, slightly rapid breathing, and slight piloerection. The highest dose group was 256 g/kg BW showed the most toxic symptoms. Toxic symptoms include feeble movement, tremors, walking with the stomach (very bent down), walking backwards slowly, rapid breathing, and piloerection. Decreasing motor activity in mice and remained silent even though they were touched. Symptoms of this toxicity occurred until the mice died.

Compared to the control group, there was an increase in the percentage change in body weight at doses of 1, 2, 4, 8, and 16 g/kg BW from the first day to the 14th day. While at doses 32, 64 and 128 g/kg BW, there was a decrease in the percentage and weight change. Pandey *et al.*[24] stated that ethanol extract of *Hydrocotyle sibthorpioides* Lam. reservoir at a specific dose could increase the appetite of mice.[24] The highest increase occurred at an 8 g/kg BW dose. However, at larger doses of 16, 32, 64 and 128 g/kg BW, the percentage of body weight decreased compared to the previous dose. These four dose groups caused a decrease in male appetite compared to the lower dose group. The administration of large doses to animals causes suffocation and possibly lesions of the hypothalamus Intilateral (as the centre of hunger).[25] Following the observation of toxic symptoms, mice began to show toxic symptoms, namely weakness at a dose of 16 to 128 g/kg BW. the proportion of weight change data for a dose of 256 g/kg BW not included because all the test animals died within 24 hours.

Based on one-way ANOVA, the administration of *Hydrocotyle sibthorpioides* Lam. extracts at various doses with a significant percentage change in body weight ($p<0.05$). DMRT results showed no significant difference between the control and treatment groups 1, 2, 4, 16, 32 and 64 g/kg BW. The significant differences occurred between the control group with a dose of 8 g/kg BW. It showed increases in the highest percentage change in body weight. While the control group, at a dose of 128 g/kg BW, showed the highest percentage change in body weight decreased.

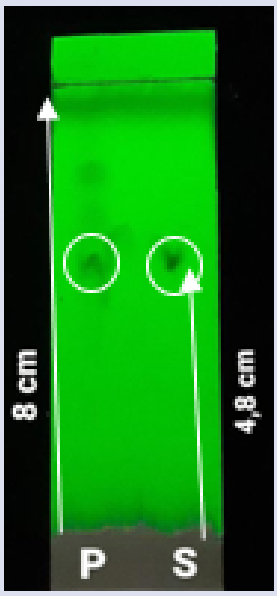


Figure 1: TLC profile of **Hydrocotyle sibthorpioides Lam.** ethanol extract at 254 nm UV light.
Note: P = comparison (routine), S = sample (extract).

Table 3: LD_{50} value determination based on Indonesia Pharmacopeia third edition.

Doses (g/kg BW)	Mice on treatment group	Death Mice	Life	Pi	ΣPi
1	10	0	10	0	
2	10	0	10	0	
4	10	0	10	0	
8	10	0	10	0	
16	10	0	10	0	1.5
32	10	0	10	0	
64	10	1	9	0.1	
128	10	4	6	0.4	
256	10	10	0	1	

LD50 value obtains by the formula below:[26]

$$m = a - b(\Sigma pi - 0.5)$$

m = log LD_{50}

a = the logarithm of the lowest dose that causes 100% of deaths in each group

b = successive dose log difference

pi = The number of animals that died after receiving the first dose, then divided by the total number of test animals that received dose i.

Table 4: LD_{50} categories.[27]

Categories	LD_{50} oral (on mice)
Super Toxic	< 5 mg/kg
Extremely toxic	5-50 mg/kg
Very Toxic	50-500 mg/kg
Moderately Toxic	500-5000 mg/kg
Slightly Toxic	5000- 15,000 mg/kg
Practically Non-toxic	>15,000 mg/kg

LD50 measurement on ethanol extract *Hydrocotyle sibthorpioides* Lam. based on Indonesia Pharmacopeia third edition. It stated that doses must be multiple constantly, the same number of mice in the animal treatment group, and doses arranged to kill the mice from 0% to 100% in one group treatment.

Based on the formula above, obtain the LD_{50} value in 128.83 g/kg BW. According to that value, the safety level of ethanol extract of *Hydrocotyle sibthorpioides* Lam. showed in Table 4. It obtains that LD50 of ethanol extract of *Hydrocotyle sibthorpioides* Lam. > 15,000 mg/kg, which means practically not toxic.

In liver histopathology, doses of 7 mg/kg BW and 35 mg/kg BW for 7th, 14th and 21st days gave a level of damage <10% (score 0) in steatosis, inflammation, necrosis, and fibrosis. Therefore, the average score obtained was 0, meaning that the liver histology was still normal, the same as the control group, and a dose of 150 mg/kg BW administered for seven days. However, giving a dose of 150 mg/kg BW for fourteen days showed damage in the form of 10-30% steatosis (score 1), inflammation 10-30% (score 1), necrosis 10-30% (score 1), and fibrosis <10% (score 1). score 0), so the average score obtained is 0.75, meaning that there is mild damage to liver histology. Giving a dose of 150 mg/kg BW for 21 days showed damage in the form of 10-30% steatosis (score 1), inflammation 31-60% (score 2), necrosis 10-30% (score 1), and fibrosis <10% (score 0), so the average score obtained is 1, meaning that there is moderate damage to liver histology. The results of liver histology observations after administration of *Hydrocotyle sibthorpioides* Lam. extract showed in Figure 2.

In kidney histopathology, administering a dose of 7 mg/kg BW for 7th, 14th, and 21st days showed normal histology. Dosage of 35 mg/kg BW for 7, 14, and 21 days showed minimal damage (degeneration <10%). Dosage of 150 mg/kg BW for 7 days showed mild damage (degeneration <25%), while doses of 150 mg/kg BW for 14 and 21 days showed moderate damage (degeneration <50%). The renal histology observations after administration of *Hydrocotyle sibthorpioides* Lam. extract showed in Figure 3.

DISCUSSION

Liver cell damage observed variously. It observed by steatosis, inflammation, necrosis, and fibrosis.

Steatosis occurs due to the diversion of normal substances away from catabolism. It leads to fat biosynthesis due to excessive formation of nicotinamide adenine dinucleotide, impaired lipoprotein formation and secretion, and increased peripheral fat catabolism.[19] This study found that steatosis damage was <10% at 7 and 35 mg/kg BW doses on the 8th, 15th, and 22nd days and the 150 mg/kg BW on the eighth day. Meanwhile, on the dose of 150 mg/kg BW on the 15th and 22nd days, there is total damage of 10-30%, which means there is light damage.

Furthermore, inflammatory cells observed the reaction of living tissue to all forms of injury in the form of a vascular reaction which results in the delivery of fluid, dissolved substances, and cells from the blood circulation to the interstitial tissue in the injured area. In this study, the number of inflammatory cells was <10% in the 7 and 35 mg/kg BW dose group on the 8th, 15th, and 22nd days. However, at a dose of 150 mg/kg BW on the 15th day, there was 10-30% inflammation, which means there is minor damage. In addition, at a dose of 150 mg/kg BW on day 22, the number of inflammatory cells increased to 31-60%, meaning that the damage was moderate. Liver damage occurs because of toxic substances in large and continuous amounts.[28] Observations also found the presence of inflammatory cells in the control group. It likely to happen because inflammatory cells are a form of defence or protection of cells against a pathogenic agent. Inflammatory cell infiltration responds to disease or toxic agents.[29] Baratawijaya[20] also reported that inflammatory cell infiltration is a protective response to maintain structure and improve tissue function.

The cell damage necrosis observed. Necrosis occurs due to too much material that must be reabsorbed by hepatocyte cells, resulting in cell death. Necrosis is an advanced stage of hydropic degeneration characterised by swelling of the cytoplasm and granular cytoplasm. It is because the cell cannot eliminate water so that it accumulates in the cell, causing the cell organelles to absorb water and swell.[30] Necrosis appears in 3 patterns: karyolysis (missing cell nucleus), pyknosis (black cell nucleus), and karyorrhexis (cell nucleus blurring or cracking).[31] This study found that the number of cell death (necrosis) <10% in the dose group 7 and 35 mg/kg BW on the 8th, 15th, and 22nd days and at a dose of 150 mg/kg BW on the 8th day. 150 mg/kg BW on the 15th day and 22 cells that experienced necrosis amounted to 10-30%, which means there was light damage. When the liver continuously exposed to drugs and chemicals in the long term, the liver cells can change, especially in hepatocyte cells, such as degeneration, which can reduce cell regeneration ability, causing cell death.[28] The description of the damaged cells shown in Figure 2.

In this study, changes in hepatocyte cells in the form of necrosis did not only occur in the treatment group but also occurred in a small number in the control group. It is bound to happen because liver cells experience physiological death (normal development). Cell death could have occurred in the control group's liver because the changes that occurred in the cells were not always a pathological condition. Test animals under normal circumstances are also likely to experience cell damage. It occurs because of several factors such as mechanical trauma, radiation, and changes in temperature and pressure.[32]

Furthermore, cell damage in the form of fibrosis observed—fibrous tissue forms in response to inflammation or direct toxic disturbances to

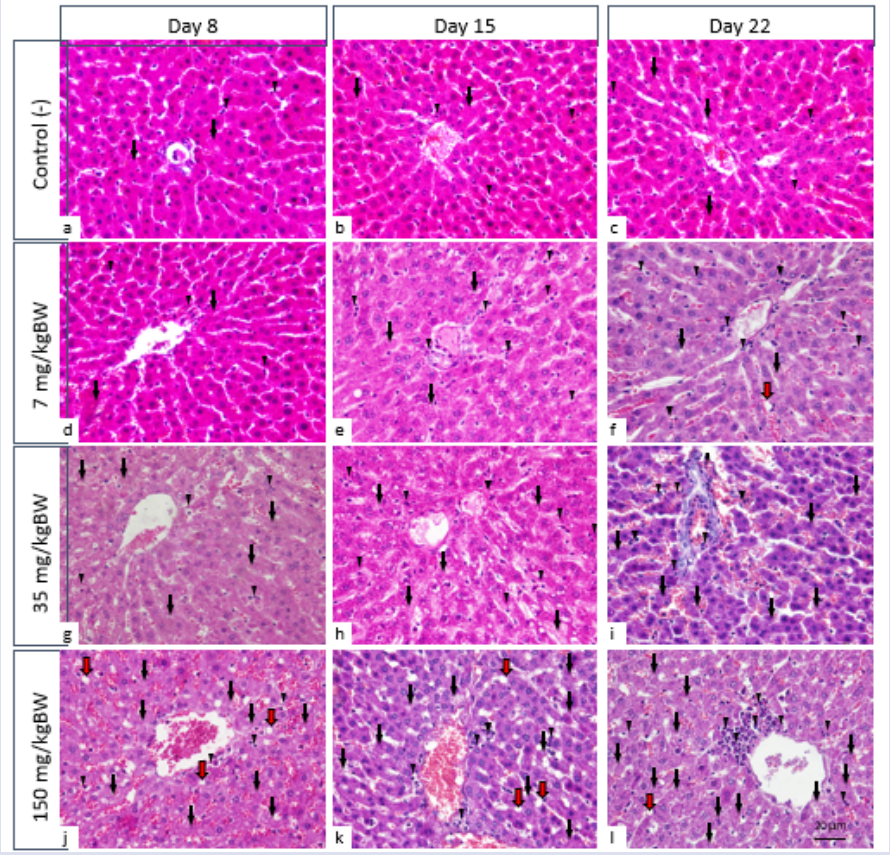


Figure 2: Histopathology of the liver. Description: necrosis (black arrow), inflammation (arrowhead), steatosis (red arrow).

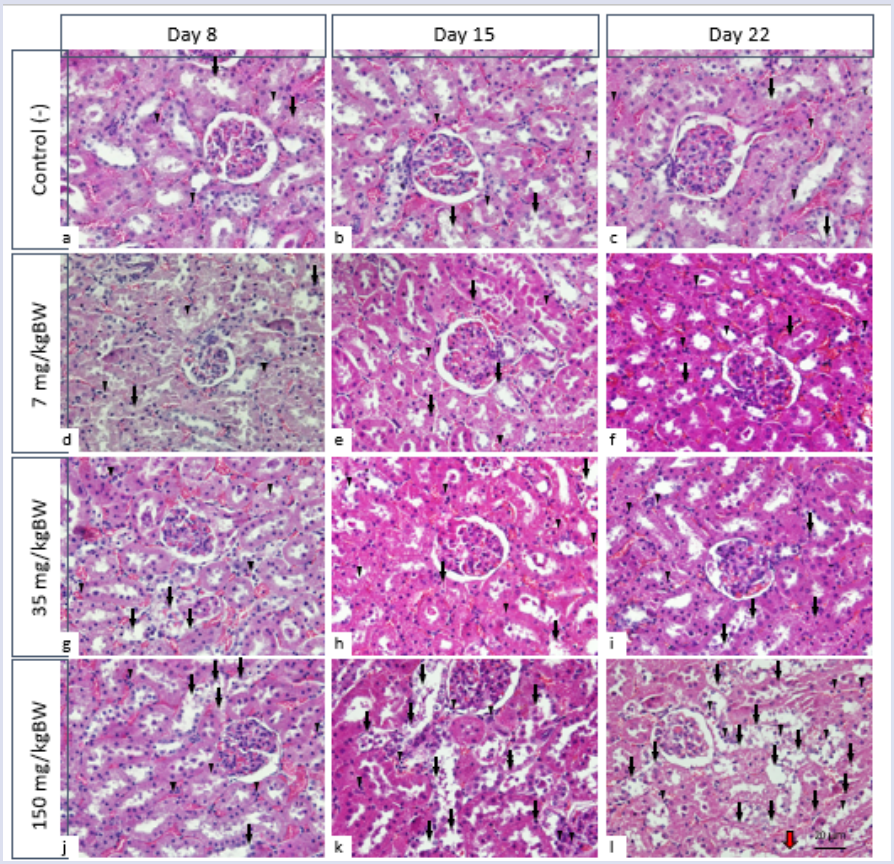
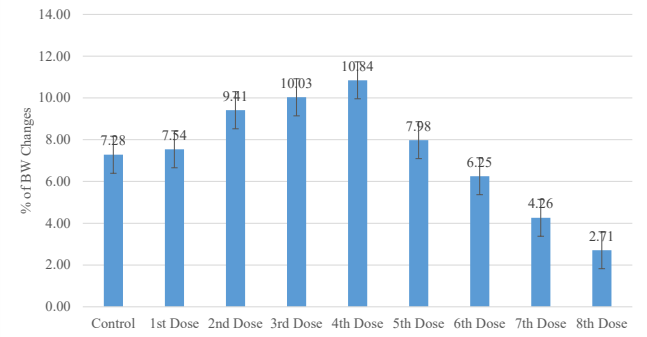


Figure 3: Kidney histopathology. Description: degeneration (black arrow), inflammation (arrowhead).



Graph 1: Correlation doses and percentages of body weight changes.

Dose explanation:

1st Dose= 1 g/kg BW	2nd Dose = 2 g/kg BW
3rd Dose = 4 g/kg BW	4th Dose = 8 g/kg BW
5th Dose = 16 g/kg BW	6th Dose= 32 g/kg BW
7th Dose = 64 g/kg BW	8th Dose = 128 g/kg BW

the liver. Initially, fibrosis forms in or around the portal tract or central vein, or it may deposit directly in the sinusoids.[19] In this study, it was unclear that there was fibrosis at all dose levels, both on days 8th, 15th, and 22nd day. If severe fatty liver accompanies inflammation or even liver cell death, it can develop in scar tissue (fibrosis) formation in the liver.[19]

The histological damage to the kidneys observed was tubular epithelial cells that experienced degeneration or necrosis. In negative control animals, the kidney tissue consisted of a cortex consisting of tubular cells and a glomerular structure. In the treated animals, especially at high doses of 150 mg/kg BW, there was an increase in damaged tubular epithelial cells, either in the form of changes in the cytoplasm with vacuolated cytoplasm or necrosis with pyknotic, fragmented or lytic nuclei. There was a slight increase in the distribution of inflammatory cells in the kidney parenchyma tissue in the treated animals, but the increase was not too significant. Kidney cell damage due to *Hydrocotyle sibthorpioides* Lam. extract shown in Figure 3.

At a dose of 7 mg/kg BW and a dose of 35 mg/kg BW, there was no apparent change in the histological structure of the kidney. There is a non-significant increase in degenerating and inflammatory cells in the parenchyma. The kidney cell damage more visible than at a 150 mg/kg BW dose, especially on days 15 and 22. According to Shecilia *et al.*,[33] types of damage such as degeneration and necrosis are damage due to chemical compounds in the kidneys. Glomerular shrinkage and Bowman's space expansion caused by the abundance of glomerular filtrate and the precipitated protein that accumulates in Bowman's space. It is due to leakage of the glomerular filter due to increased capillary permeability so that more protein escapes to fill the Bowman's space.

CONCLUSION

Ethanol extract of *Hydrocotyle sibthorpioides* Lam. LD_{50}>15,000 mg/kg BW with safety level on practically not toxic. It has a significant effect on mice body weight changes and mice behaviour. Based on the results of the study, it concluded that the administration of extract of *Hydrocotyle sibthorpioides* Lam. at doses of 7, 35, and 150 mg/kg BW for the 7th, 14th, and 21st days did not show severe damage to the histology of the liver and kidneys of male mice. It concluded that the *Hydrocotyle sibthorpioides* Lam. are safe and did not cause damage to the liver and kidney.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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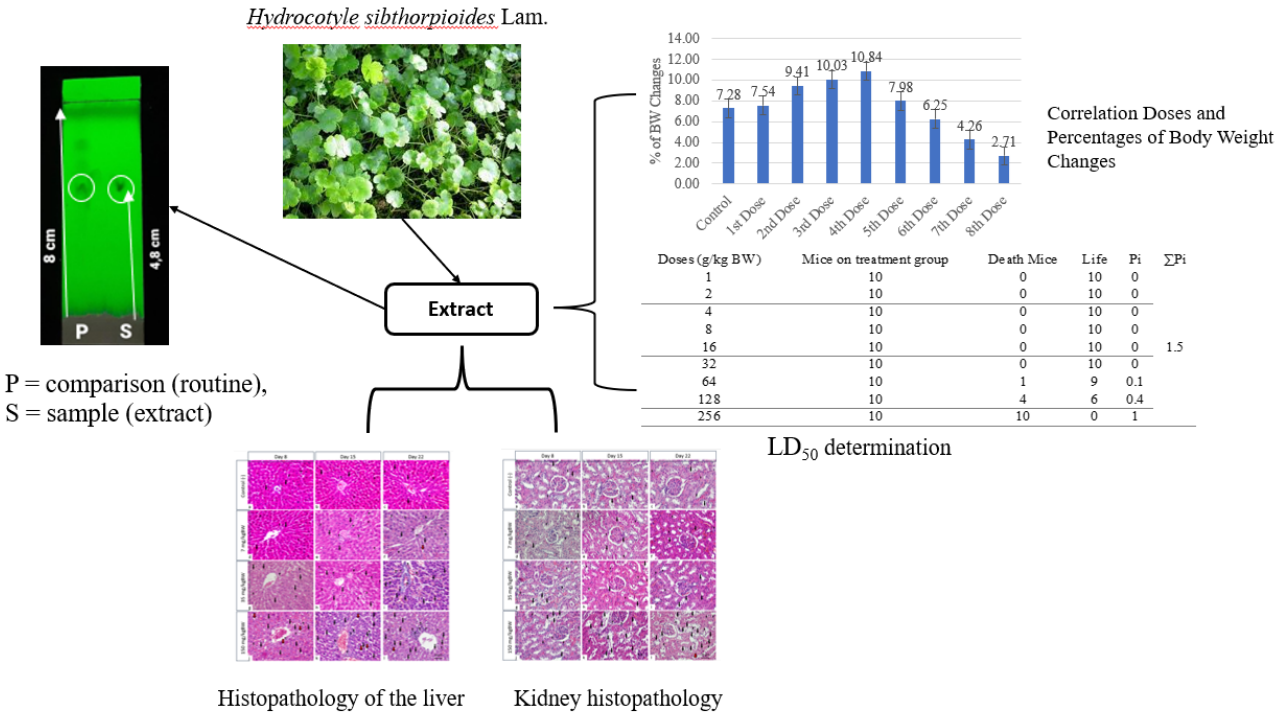
GRAPHICAL ABSTRACT



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