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Acute and Sub-Acute Oral Toxicity Assessment of *Marchantia paleacea* Bertol. Liverwort Herb Extract in Mice on Liver and Kidney Function

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ABSTRACT

The Liverwort herb, *Marchantia paleacea* Bertol. (M. paleacea), which has a variety of bioactive compounds, offers pharmacological benefits such as: antimicrobials, antioxidants, immunomodulators, hepatoprotectors, and diuretics. However, data on acute and sub-acute oral toxicity from this plant ethanol extract are still limited. This study aims to evaluate the acute and sub-acute toxicity of this liverwort ethanolic extract in female mice of ddY strain. The acute toxicity test was carried out with a single dose of 250, 500, 1000, and 2000 mg/kg bw for 14 days, while the sub-acute toxicity test with a dose of 125, 250, 500, 1000, and 2000 mg/kg bw for 28 days, according to BPOM (2022), Thompson-Weil, and OECD standards. The parameters observed included clinical symptoms, weight changes, organ index, biochemical analysis, liver and kidney histopathology. The results of acute toxicity tests showed that a single dose of EEMP did not cause clinical symptoms or significant histopathological disorders, nor were there any deaths in the test mice. Sub-acute toxicity tests showed significant increases in SGOT at doses of 125, 250, and 2000 mg/kg bw on day 28, but still within normal limits. Most of the other parameters did not show significant changes. Some of the changes observed are thought to be temporary reactions, not indications of toxicity. This study adds insight into the potential use of *M. paleacea* Bertol. as an herbal product, as well as the importance of understanding the risk of toxicity that may be involved.

INTRODUCTION

Medicinal plants are plants that are useful for medicines, cosmetics, nutraceutical, and health supplements consumed or used from plant parts such as leaves, stems, fruits, tubers, rhizomes, roots, or whole parts (herbs). The use of herbal preparations is accepted in almost all countries of the world. In developed countries, the use of certain herbal remedies is very popular. In developing countries, the majority of the population still uses herbal medicines, especially for basic health needs and traditional medicines as complementary and/or alternative medicines with/without conventional drugs (herbal medicine as an alternative monotherapy or in combination with conventional medicine). Because they have relatively lower side effects, traditional medicines have also been widely

accepted and used in almost all countries in the world (Fatirah *et al*., 2019a; Siregar *et al*., 2020).

The driving factor for the use of traditional medicine is because conventional (modern/synthetic) treatment is considered to be able to fail in treatment (Fatirah *et al*., 2019a; Siregar *et al*., 2020). *Marchantia paleacea* Bertol. (M. paleacea) is a species of liverwort that belongs to the Marchantiaceae family. This liverwort species has been utilized as a botanical treatment in a multitude of traditional remedies for several medical conditions, but there are no preclinical and clinical scientific data related to acute and sub-acute toxicity data of the herb (M. paleacea). It is imperative to comprehend the potential ramifications of the herb's toxicity, particularly in the context of its application inside human healthcare (Atwood and Buck, 2020; Purkon *et al*., 2021a; Puspitasari *et al*., 2021). Liverworts of the genus Marchantia are already used as a folk remedy that has long been used in China, Europe and North America (Fadhilla *et al*., 2012). The genus of Marchantia can traditionally be used to heal cuts, fractures, viper bites, burns, blisters, and open wounds (Wang *et al*., 2016). Various pharmacological activities were observed from liverwort extracts of (M. paleacea) species, namely: antimicrobial, antifungal, antioxidant, antipyretic, diuretic, cytotoxic and apoptotic activity, cardiotonic, muscle relaxant, immunomodulatory, and antihepatitis (Purkon *et al*., 2022b, 2022a, 2021b).

Thorough toxicity testing is imperative prior to the use of a component in the field of human medicine. Toxicity testing plays a crucial role in establishing appropriate dosage levels and detecting any adverse reactions (Fakri *et al*., 2020; Greco *et al*., 2020; Warsito, 2021). Despite the intriguing pharmacological potential of liverwort herb, there remains a scarcity of research regarding its toxicity. Hence, it is imperative to do research on the acute and sub-acute toxicity orally in animal models, specifically mice, in order to address this gap in information for *Marchantia paleacea* Bertol. extract (Simmons and Herman, 2023).

The utilization of botanical substances in medical practice is experiencing an increase in popularity, leading to the emergence of herbal pharmacology as a burgeoning academic discipline. Hence, the investigation into the toxicological properties of herbs holds considerable significance within the realm of contemporary pharmacology. This research is expected to provide new insights regarding the safety of the use of the herb (M. paleacea) in medicine and nutraceutical, as well as the potential toxic effects at large doses that may occur. The results of this study can also be used as a basis for further research on this test plant (Purkon *et al*., 2022b; Xue *et al*., 2023).

The ddY strain of mice is frequently employed as an animal model in the field of toxicity research. The examination of toxicity in ddY mice can yield valuable insights into the potential risks of toxicity in humans (BPOM, 2022; Darmawan *et al*., 2020; OECD, 2008; Saleem *et al*., 2017). Numerous nations have implemented regulatory frameworks pertaining to herbal medicines, necessitating the conduction of toxicity assessments prior to the commercialization of such goods. Accordingly, this study holds significance within the domain of pharmaceutical regulation (Ng *et al*., 2022).

METHODS

Population and Test Samples

The research conducted is true experimental research that refers to standards or methods from the Food and Drug Supervisory Agency (BPOM, 2022), Thompson-Weil, and OECD (BPOM, 2022; OECD, 2001). Based on these three references, namely: the BPOM (2022), Thompson-Weil, and OECD (2021) methods both use rodent test animal models, such as: mice in acute and sub-acute toxicity tests. The Thompson-Weil method emphasizes on the measurement of detailed preclinical (clinical), biochemical, and histopathological parameters for the process of assessing the toxicity effect. The BPOM (2022) and OECD (2021) methods emphasize the number of test animal replication, validation of results, and interpretation of globally acceptable data (BPOM, 2022; OECD, 2001).

Fresh herbal samples of liverwort *Marchantia paleacea* Bertol. were obtained or harvested from the areas of Padajaya Village and Sindangjaya Village, located in the Cipanas District of the Cianjur Regency in West Java, Indonesia. While the rodents used were female mice of *ddY* strain obtained from the Faculty of Veterinary Medicine, Bogor Agricultural University (IPB), Indonesia.

Place and Time of Research

This research was conducted at the Pharmacology Laboratory and Phytochemistry Laboratory of the Department of Pharmacy Poltekkes Kemenkes Bandung, Integrated Laboratory Poltekkes Kemenkes Bandung, and Laboratory of Medical Laboratory Technology Department of Medical Laboratory Technology

(TLM) Poltekkes Kemenkes Bandung. The period of this research was from January to July 2023.

Tools and Materials Used

The test materials used in the extraction process, the manufacture of test preparations, the testing process, and assisting in the observation in the test, are: 96% solvent ethanol, aquadest, chloroform, HCl, NaCl, Dragendorff reagent, Liebermann-Burchard reagent, Mayer reagent, Mg powder, ether, anhydrous H_2SO_4, concentrated H_2SO_4, $FeCl_3$, NaOH, Na-CMC, gelatin, and 10% formalin.

Meanwhile, the various types of tools used, namely: surgical instruments, surgical boards, mice test animal drums, masks, gloves, mice scales (Tanita KD-160), analytical scales (Sartorius BL 210), oral sonde, syringe, surgical instrument set, watch glass, vaporizer dish, platinum crucible, furnace, desiccator, oven, mouse drinking bottle, surgical scissors, mortar and stamper, drip plate, bunsen heater, macerator device, electric heater, stopwatch, blacu cloth, tricycle, gauze, spray bottle, drip pipette, volume pipette, measuring flask, test tube, metal spatel, waterbath, and glass funnel.

Simplisia Setup and Test Extract

Herb liverwort *Marchantia paleacea* Bertol. collected/harvested and then cleaned with running water and dried by aerating (not directly exposed to sunlight) for 3-7 days. After drying, the test simplicia is weighed and mashed so as to obtain a powder-shaped simplicia. Then, simplicia is put into a tightly closed container and stored at room temperature.

Marchantia paleacea Bertol. liverwort simplisia powder, which is already in powder form, then is used in the extraction process by maceration by mixing 600 g of simplicia powder with 2.4 L of 96% ethanol. The extraction process is carried out for 72 hours or 3 days (every 24 hours a replacement is carried out with a new solvent and a stirring process is carried out periodically). Then the product undergoes a filtering process and the evaporation process with a rotary vacuum evaporator device at 60oC. The thick extract obtained is then undergoes the process of concentrating the viscous extract with a waterbath tool until a constant weight is obtained (the difference in weighing is not more than 0.05 mg). The extract is stored in containers, sealed, and stored at 4^{o}C (Fadhilla *et al*., 2012; Purkon *et al*., 2022b, 2021a).

Simplicia Test Drying Shrinkage Inspection

The test cup (empty) is heated using an oven at 105^{o}C for 1 hour. Then the empty test cup is inserted into the desiccator for 10-15 minutes, then weighed with the weight of the empty test cup using an analytical balance. After that, 1 g of test simplicia sample is inserted into the cup and the weighing process is carried out. The test cup that has contained the simplicia is then heated into the oven for 5 hours with a temperature of 105^{o}C. After the heating process is complete, then the cup containing the test sample is inserted into the desiccator for 30 minutes and after that the weighing process is carried out for the cup containing the test sample (Depkes RI, 2000; Kemenkes RI, 2017, 2014).

Phytochemical Screening Test and Suspension Preparation of Ethanol Extract of Liverwort Herb *Marchantia paleacea* Bertol. (EEMP)

The EEMP obtained then undergoes a phytochemical screening process for the examination of alkaloid group compounds, flavonoids, saponins, terpenoids and steroids, polyphenols, and tannins in accordance with the procedures of the Standardization Parameters of Plant Extracts and/or Indonesian Herbal Pharmacopoeia (Depkes RI, 2000; Kemenkes RI, 2017, 2014).

First, Na-CMC is weighed 1 g with an analytical balance. An aqueous solvent was also prepared as much as 10 mL in the measuring flask. Then the aqueous solvent is put into a mortar and after that, Na-CMC is sprinkled on the aqueous solvent little by little to form a thin layer. Then the grinding process is carried out until it becomes mucilago. The musilago is then added aqueous solvent again up to 100 mL. It is then stirred/shaken until homogeneous, and then put into the container and marked.

Oral Acute Toxicity Testing (14 Days)

The process of testing acute (14 days) and sub-acute (28 days) toxicity per oral preclinically (*in vivo*) has been approved by the Health Research Ethics Committee (KEPK) Poltekkes Kemenkes Bandung with No. 24/KEPK/EC/V/2023. Female mice of *ddY* strain were acclimatized/adapted for 7 days in the test animal drum to adjust to the environment of the test animal. The acclimatization process for 7 days before the test treatment aims to make the test animals accustomed to the test condition environment and not stressed (Sinam *et al*., 2016). The test animals must be satisfied before treatment for +/- 3-4 hours while still being

given drinking water. After fasting, test animals are weighed and given test preparations. The test preparation was given with 4 dose levels, namely: EEMP (ethanol extract of liverwort herb *Marchantia paleacea* Bertol.) 250, 500, 1000, and 2000 mg/kg bw then observed symptoms of acute toxicity for the first 4 hours and then for 24 hours, and observed behavior, organ (liver and kidney) indexes (% w/w), histopathology, and counted the number of mice that died from each test group, observation continued for 14 days (without giving test preparations). LD_{50} is calculated according to standards from BPOM (2022) and OECD methods (BPOM, 2022; Norazlina *et al*., 2013; OECD, 2001; Yahaya *et al*., 2021).

Percentage of Organ (Liver/Kidney) Indexes

$$(\%\ w/w) = \frac{Organ\ Weight\ (g)}{Body\ Weight\ of\ Mice\ (g)}\ x\ 100\%$$

Oral Sub-Acute Toxicity Testing (28 Days)

Oral sub-acute toxicity testing is carried out in accordance with guidelines from BPOM (2022) and OECD. Female mice of ddY strain were randomly divided into 6 test groups (n=5 per group), consisting of normal control group (group given preparation or carrier compound Na-CMC 1%), EEMP group dose 125, 250, 500, 1000, and 2000 mg/kg bw. All test groups were given their own test preparations every day for 28 days of testing. All test animals were carefully observed during the first 1 and 4 hours after administration to check for signs of adverse toxicity, behavioral changes, weight change profile, and clinical biochemical levels of vital organs, such as liver function (SGPT and SGOT levels), and kidney function (creatinine level). The body weight of test animals is weighed and evaluated periodically. On the 29th day, after the test animals were satisfied at night for approximately 8 hours, then all test animals were euthanized and underwent intracardiac blood collection procedures. The blood is then allowed to stand for 10 minutes, then a centrifugation process is carried out at 3000 rpm for 10 minutes, and the blood serum is separated to be examined for clinical biochemical levels of vital organs, namely liver and kidney function or stored blood serum at freezer temperature (< -20oC) for biochemical testing, such as: SGPT and SGOT levels to see the function of the liver organs and creatinine levels to see the function of the kidney organs (BPOM, 2022).

Statistical Analysis

The data collected in this study involve quantitative data. The quantitative data to be obtained are the number of experimental animals that died. LD50 data also were taken from the number of female mice of ddY strain that died in each test group. Furthermore, the LD50 value was calculated using the Thompson-Weil method and BPOM (2022) (BPOM, 2022; Kumarnsit *et al*., 2021; OECD, 2001; Ofori *et al*., 2021; Roy *et al*., 2016; Vadakkan, 2019).

RESULTS AND DISCUSSION

Samples of liverwort herb test plants *Marchantia paleacea* Bertol. underwent the process of determination/identification of test plants at the Plant Taxonomy Laboratory at the Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Universitas Padjadjaran with No. 36/HB/05/2023 which showed that the test plants were *Marchantia paleacea* Bertol. species. The fresh herbs as much as 10 kg then underwent a wet sorting process with the aim of separating particles and other impurities attached to the fresh herbs. The washing process is done using running water to remove dirt that is still attached. Then the drying process is conducted by aerating the method indoors until simplicia (dry matter) is obtained from the test herbs. After that, a dry sorting process is carried out until 3 kg of simplicia is obtained. The test simplicia obtained was then determined by 3 times of replication of drying shrinkage, until an average percentage of drying shrinkage of 7.26% w/w was obtained. The simplicia obtained is then carried out an extraction process by maceration method (cold way extraction) using 96% ethanol solvent. The viscous extract with a constant weight was produced as much as 25.083 g with an extract yield percentage of 4.18% w/w.

Organoleptic examination of the test extract includes: the form of the extract in a thick state, smells typical of aromatics, tasteless which over time will cause a chelate taste, and blackish brown. In the results of phytochemical screening from EEMP test extracts, data were obtained that ethanol test extracts of this liverwort herb *Marchantia paleacea* Bertol. contain flavonoid group compounds, saponins, tannins/polyphenols, and terpenoids/steroids.

EEMP Effects of Observations and General Habits in Female Mice Test Animals *ddY* Strains for Acute Toxicity Test for 14 Days and Sub-Acute Toxicity Test for 28 Days

In oral acute toxicity testing, all female mice of *ddY* strains from various test group treatments were given their respective test preparations which were then observed for signs of acute toxicity which included: the

presence/absence of behavioral changes, significant changes in motor and autonomic activity compared to normal control groups, or even death (mortality) in test animals for 4 hours, the first 24 hours, and for 14 consecutive days as can be observed in Table 1.

The profile of the average percentage change in body weight of female mice of *ddY* strain (%) in acute toxicity tests on days 1, 7, and 14 and sub-acute toxicity tests on days 1, 7, 14, and 28 with their respective test dose groups can be seen in Figure 1.

This was done to see the effect of giving test extracts on the profile of weight changes that occurred (g) for 14 days (in acute toxicity tests) and 28 days (in sub-acute toxicity tests). All acute toxicity test groups experienced an increase in average body weight on day 14 compared to day 1, especially in the EEMP -

Table 1. Data from the Observation of Acute Toxicity Test Perorally for 14 Days Related to Behavior, Motor Activity, and Mortality from the Herb Test Extract of Liverwort *Marchantia paleacea* Bertol. (n = 5)

No.	Test Group	Laccrimation	Salivation	Tremor Event	Seizure Events	Grip Strength	Drowsiness	Eat	Drink	Mortality
1.	Normal Control (NC)	Normal	Normal	Not happening	Not happening	Normal	Normal	Normal	Normal	0
2.	EEMP Dose 250 mg/kg bw	Normal	Normal	Not happening	Not happening	Normal	Normal	Normal	Normal	0
3.	EEMP Dose 500 mg/kg bw	Normal	Normal	Not happening	Not happening	Normal	Normal	Normal	Normal	0
4.	EEMP Dose 1000 mg/kg bw	Normal	Normal	Not happening	Not happening	Normal	Normal	Normal	Normal	0
5.	EEMP Dose 2000 mg/kg bw	Normal	Normal	Not happening	Not happening	Normal	Normal	Normal	Normal	0

Information:
EEMP = Ethanol extract of the liverwort herb *Marchantia paleacea* Bertol.
0 = No deaths (or 0 animals died)

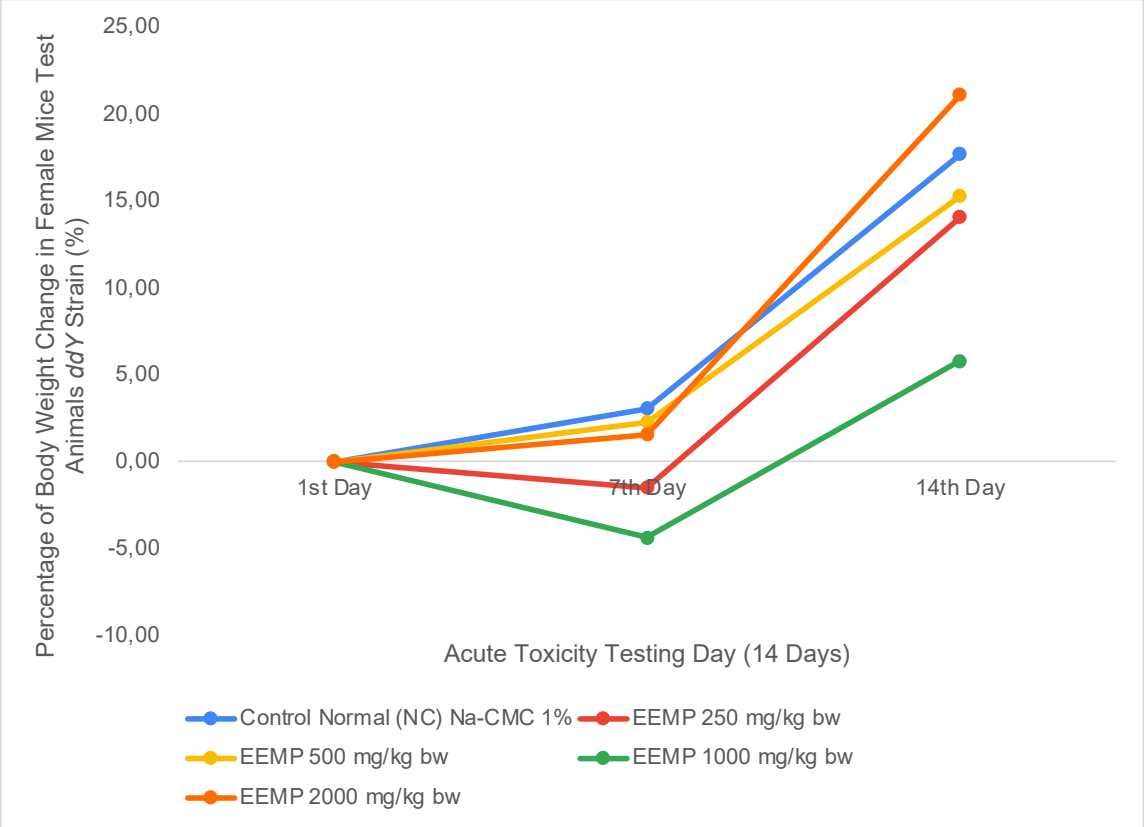
test group dose of 2000 mg/kg bw up to 21.09%. While on day 7 there was a decrease compared to day 1 in the EEMP group dose of 1000 mg/kg bw but not more than 20% as seen in Figure 1 (a).

This was done to see the effect of EEMP test extract on the profile of weight changes that occurred during the 14-day period of acute toxicity massage. Conditions that indicate that the test animal is experiencing pain usually appear when the body weight decreases by more than 20% for at least 7 days or more. Usually associated with a lack of food intake that can be due to internal factors from test animals. Test animals experienced fluctuating weight changes indicating illness or suffering after giving the test extract (Turner *et al*., 2019).

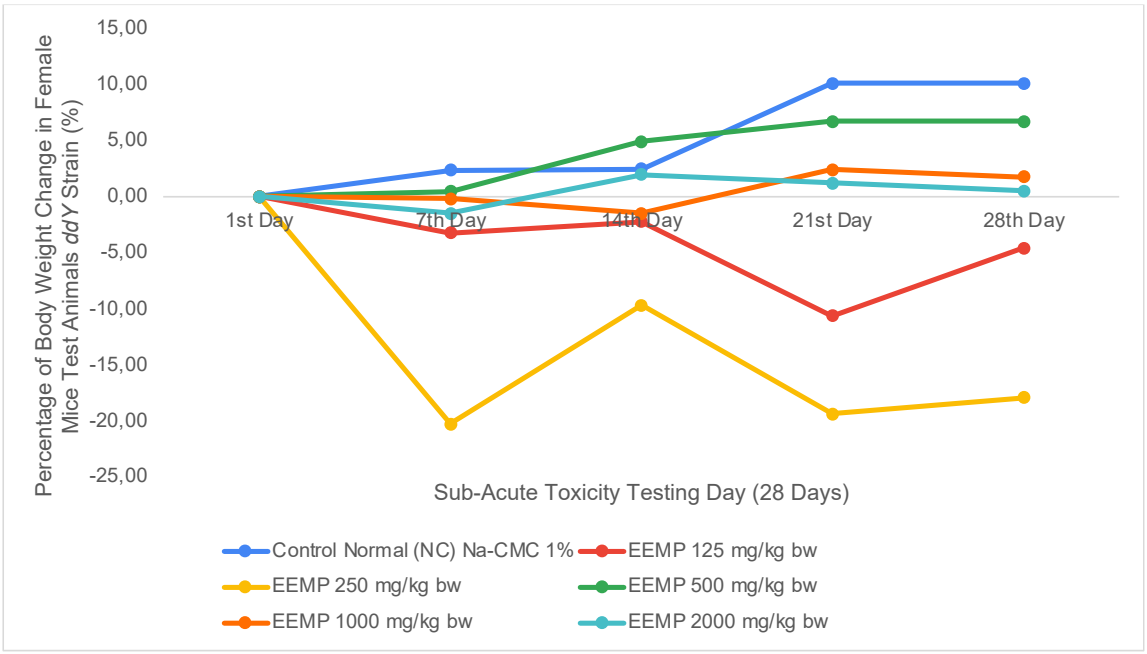
EEMP Effect of Liver and Kidney Index Results (% w/w) As Well As Histopathology Assessment on Both Organs in Oral Acute Toxicity Testing For 14 Days

The percentage index of liver and kidney organs (% w/w) as vital organs can be seen in Table 2. In observing the liver and kidney index data (% w/w) there was no significant difference compared to normal control (NC) based on the statistical results of the one-way ANOVA and post-hoc LSD tests.

The calculation of the liver and kidney index (% w/w) is to determine the toxic effects on internal vital organs that occur after the administration of test preparations (Djohari *et al*., 2022; Fatirah *et al*., 2019b). The data from the analysis showed that the average kidney weight of test animals in all groups was still within normal limits. In research conducted by Parmar and Prakash (2006), it was explained that the normal range of the kidney organ index of mice test animals is (≤ 1.6%). In addition, in the EEMP test group with 2000 mg/kg bw, there was a decrease in organ index in liver organs -



(a)



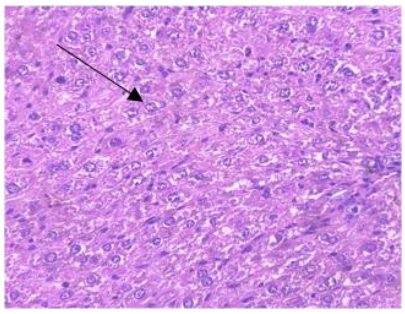
(b)

Figure 1. Percentage graph of average profile of weight change in female mice of *ddY* strain (%) in acute toxicity test on days 1, 7, and 14 (a), and percentage profile of average weight change in female mice of *ddY* strain (%) in sub-acute toxicity test animal on days 1, 7, 14, 21, and 28 (b) (n=5)

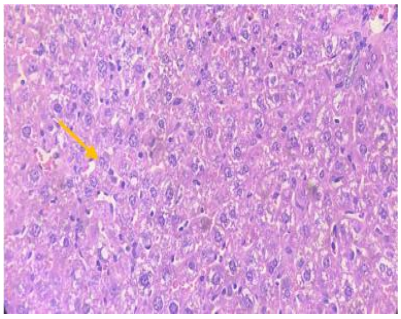
Table 2. Liver and Kidney Index Data (% w/w) in all Peroral Acute Toxicity Test Groups in Female Mice of *ddY* strains at Day 14 (n = 5; $\bar{x} \pm SD$)

No.	Test Group	Index Organ (% w/w)	
		Liver	Kidney
1.	Normal Control (NC)	5.83 ± 1.2660	1.32 ± 0.1790
2.	EEMP Dose 250 mg/kg bw	6.33 ± 0.7990	1.21 ± 0.1680
3.	EEMP Dose 500 mg/kg bw	6.43 ± 0.5999	1.34 ± 0.0356
4.	EEMP Dose 1000 mg/kg bw	6.48 ± 1.5066	1.29 ± 0.4258
5.	EEMP Dose 2000 mg/kg bw	5.67 ± 1.3834	1.20 ± 0.1974

Information: EEMP = Ethanol extract of the liverwort herb *Marchantia*



(a)



(b)

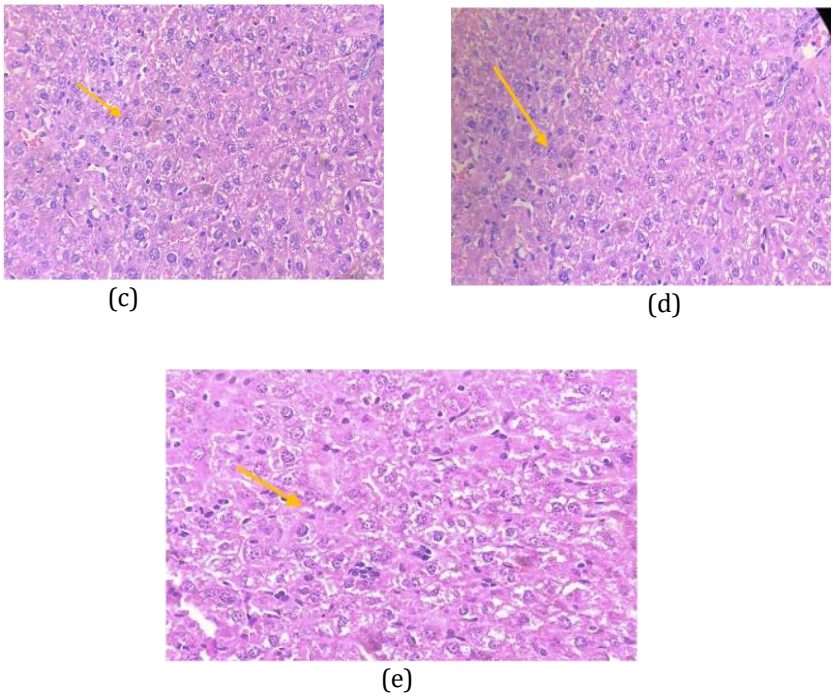


Figure 2. Histopathological examination of the liver from oral acute toxicity testing as a vital organ function with a light microscope at 400x magnification in all test groups with hematoxylin-eosin (HE) staining, which includes: (a). Normal group with black pointer marks which are normal liver cells (hepar) with a clearly visible cell nucleus (nucleus) and no cell damage, (b). Dose group 250 mg/kg bw with yellow pointer marks indicating liver cells that experience cloudy swollen degeneration which is reversible, (c). The dose group is 500 mg/kg bw with a yellow pointer indicating liver cells that experience cloudy swollen degeneration that is reversible, (d). The dose group is 1000 mg/kg bw with a yellow indicator indicating liver cells that have degenerated turbid swelling which is reversible, and (e). The dose group is 2000 mg/kg bw with a yellow indicator indicating that the liver cells have a cloudy swollen degeneration that is reversible

(5.67 ± 1.3834 % w/w) and kidney organs (1.20 ± 0.1974 % w/w) which was slightly lower than normal control (liver organ index = 5.83 ± 1.2660 % w/w and kidney organ = 1.32 ± 0.1790 % w/w) as seen in Table 2. Although the decrease occurred in the EEMP test group with a dose of 2000 mg/kg bw compared to the normal control group, it was not significant (still in the range of 1.6%) and did not cause severe toxicity. This insignificant difference may be due to the antioxidant-protective effects of the *Marchantia paleacea* Bertol plant extract and the ability of the test animals' bodies to compensate for mild biochemical changes (Raeeszadeh *et al*., 2022; Siregar *et al*., 2021).

Determination of liver and kidney index (%) was done to see the damage from increasing the test dose on the two vital organs that have very important functions for our body, namely the body's metabolic processes (liver), detoxification (liver), parts of the endocrine system (liver), hormonal (liver and kidney), and metabolite excretion processes through urination (kidney) compared to normal control groups (Parmar and Prakash, 2006). This showed that the administration of EEMP extract in macroscopically observed test animals did not cause excessive toxic effects on the weight of the kidney and liver organs of test animals.

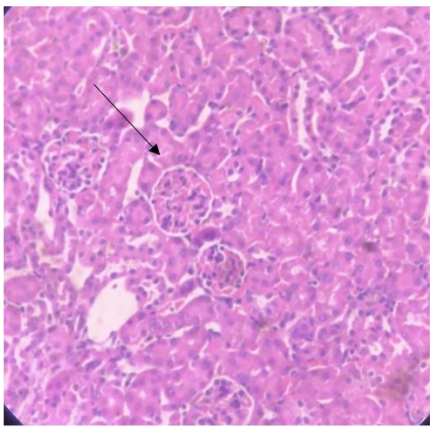
Liver and kidney organs in female mice of ddY strain that have been weighed with analytical scales from the results of acute toxicity tests, then microscopic observations (histopathology) were made on organ preparations that had been stained hematoxylin-eosin (HE). After that, the observation process is carried out using a light microscope at 400x magnification as seen in Figures 2 and 3. Both organs were then observed by looking at the damage that occurred and compared with the normal control group. In the liver, all test groups experienced reversible cloudy swelling degeneration, except for the normal control group (NC). Degenerative changes such as cloudy

swelling degeneration and hydropic degeneration are types of cell changes whose processes are reversible or can return to normal if the stimulation that causes cell damage is stopped. Parenchyma degeneration is caused by water deposits in the cell which results in oxidation failure, so that the transport of proteins produced by ribosomes is inhibited and cells will experience swelling, turbid cytoplasm there are granules due to deposits protein (Yana and Budijastuti, 2022).

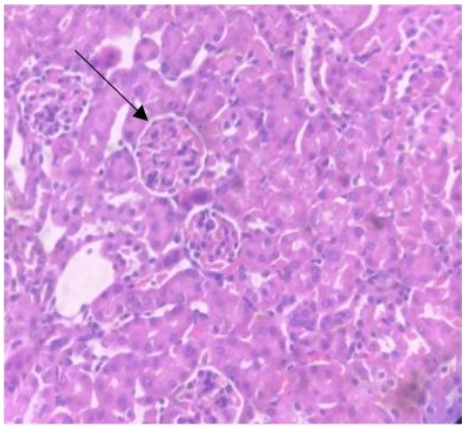
Observations of the kidney organs for EEMP doses of 250 and 500 mg/kg bw have shown that glomerular cells are normal as seen in Figure 3. While at doses of 1000 and 2000 mg/kg bw, liver cells experience glomerular atrophy. Glomerular atrophy is an event of decreased tissue size caused by a decrease in the number of cells or a decrease in cell size (Sari *et al*., 2019). *Marchantia paleacea* Bertol. liverwort herb contains flavonoid group compounds, saponins, and marchantin compounds which from various previous scientific research results have been known to have properties as antioxidants. However, an imbalance in the number of antioxidants and free radicals can cause oxidative stress that can lead to glomerular atrophy (Kamory *et al*., 1995; Pasupuleti *et al*., 2020; Purkon *et al*., 2022a; Wang and Zennadi, 2020).

This acute toxicity test was conducted to see the clinical symptoms and level of toxicity effects of the ethanol extract of the liverwort herb *Marchantia paleacea* Bertol. (EEMP) by looking at the LD_{50} value of the EEMP test sample. This acute toxicity test was conducted by the BPOM (2022) and Thompson-Weil method preclinically (in vivo) on a test animal model of female mice of the ddY strain, which is a commonly used method because it does not require a large number of test animals and has a very high level of accuracy. According to the Peroral Acute Toxicity Test Guidelines from the Food and Drug Administration/BPOM (2022) which explains that there are 4 types of rodents that can be used in oral acute toxicity testing, namely: mices, rats, rabbits, and guinea pigs. The reason for choosing the species of test animals for ddY strain mice is because mice have advantages such as a large number of offspring per birth, ease of handling, reproductive characteristics similar to other mammals, and anatomical, physiological, biochemical blood, and genetic structures similar to humans (BPOM, 2022; Mutiarahmi *et al*., 2021).

Observational results for LD_{50} values in the test group given EEMP stratified showed no clinical signs of toxicity and no mortality at the four doses of acute toxicity test administered. Various observations from acute toxicity tests of ethanol extracts of liverwort herb *Marchantia paleacea* Bertol. (EEMP) which includes behavioral parameters, motor activity, weight change profile, liver and kidney index (%), and histopathological examination up to a dose of



(a)



(b)

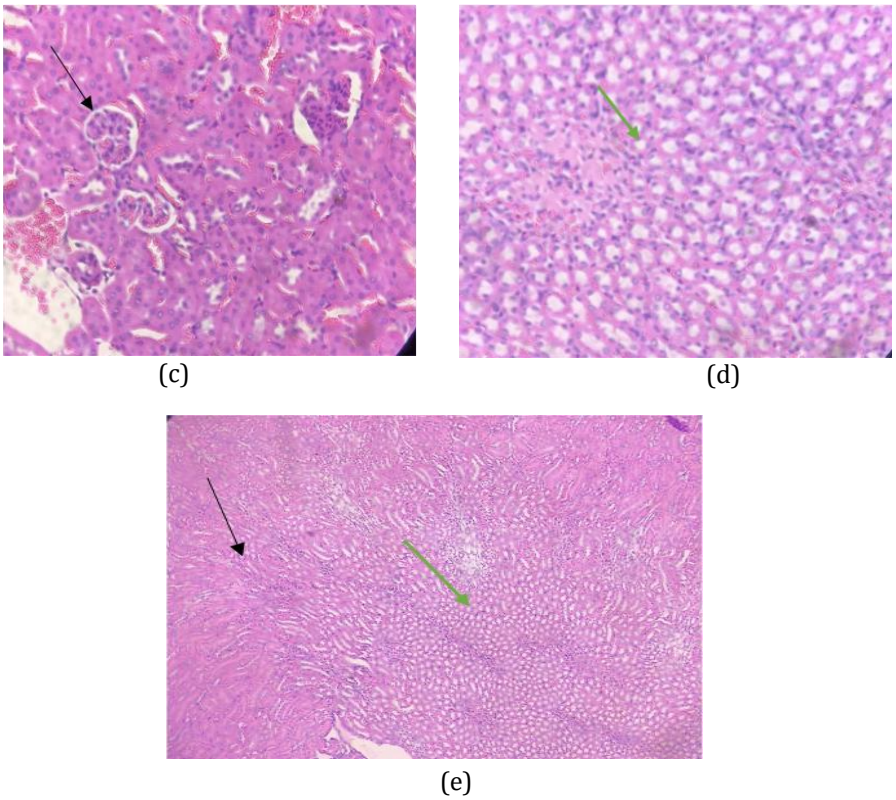


Figure 3. Histopathological examination of kidney organs from the results of acute toxicity test orally with a light microscope at 400x magnification in all test groups with hematoxylin-eosin (HE) staining as seen in: (a). Normal control with black pointer marks on kidney cells with normal glomerulus, (b). EEMP group dose 250 mg/kg bw with black pointer marks on kidney cells with normal glomerulus, (c). EEMP group dose 500 mg/kg bw with black pointer marks on kidney cells with normal glomerulus, (d). EEMP group dose 1000 mg/kg bw with green pointer mark on kidney cells with glomerular atrophy, and (e). EEMP group dose 2000 mg/kg bw with black mark on the left indicating normal glomerulus and green indicator mark on the right indicating glomerular atrophy

2000 mg/kg body weight found no symptoms of harmful toxicity during 14 days of observation. If no test animal dies, according to the LD50 Determination Table, data according to the Thompson-Weil method cannot be counted. This is in accordance with the criteria for acute toxicity tests conducted to assess LD50 that based on the agreement taken by experts in the field of toxicology, that is, if the maximum dose given does not cause death of test animals, then LD50 is expressed with pseudo LD50 or not real LD50 (Sulastra *et al*., 2020). So, if there is no death of test animals at the highest dose, namely at 2000 mg/kg bw, according to BPOM (2022), the test preparation is included in the mild toxic category (BPOM, 2022).

The process of administering EEMP test preparations in sub-acute (sub-chronic) toxicity testing orally is carried out every day for 28 days of testing time. This according to BPOM (2022) is done because it considers the possibility of a vital organ from the rodent test animal model whose regeneration process is fast and in order to maintain the concentration of test preparations in the body of the test animal in a steady state so that the toxicity effect can be observed. The observation process carried out includes toxic symptoms and clinical symptoms in the form of changes in the skin, feathers, eyes, mucous membranes, secretions, excretions, autonomic activities (for example: lacrimation, pyloerection, a state of pupils that shrink or dilate, unusual breathing patterns, changes in walking, strange behavior (for example: walking backwards), seizure events, and so on carried out every day for 28 days of observation (BPOM, 2022).

According to the WHO, clinical biochemical examination of vital organs in testing, including: liver function (SGPT, SGOT, Gamma GT/GGT) and kidney function (urea nitrogen/uric acid, creatinine, and total bilirubin) (BPOM, 2022; OECD, 2001).

Effect of EEMP on Preclinical Biochemical Parameters of Liver and Kidney Function from Oral Sub-Acute Toxicity Test for 28 Days of Testing

The results of various clinical biochemical tests on liver and kidney function can be seen in Table 3. Oral administration of EEMP test preparations in all EEMP test groups did not cause significant changes in the biochemical parameters of SGPT (ALT) and blood creatinine compared to the normal control group (NC). However, in the biochemical parameters of SGOT (AST) there were 3 doses of EEMP test, namely doses of 125, 250, and 2000 mg/kg bw which experienced a significant increase in SGOT levels compared to the normal control group (p<0.05 and p<0.01). SGPT (Alanine Aminotransferase or ALT) and serum blood creatinine levels that were still within normal ranges and did not significantly differ from the normal control group.

Table 3. Average Biochemical Results of SGPT (U/L), SGOT (U/L), and Creatinine (U/L) in Blood Serum of All Test Groups on Sub-Acute Toxicity Testing on Day 28 ($\bar{x}$ ± SD; n = 5)

Testing Group	Average Blood Serum Biochemical Levels of Test Animals on Day 28 ($\bar{x}$ ± SD)		
	SGPT (U/L)	SGOT (U/L)	Creatinine (U/L)
Control Normal (NC)	27.33 ± 1.6072	44.33 ± 5.0332	0.63 ± 0.3785
EEMP Dose 125 mg/kg bw	34.33 ± 9.2511	77.50 ± 20.9702*	0.43 ± 0.4932
EEMP Dose 250 mg/kg bw	21.33 ± 4.4814	85.50 ± 20.6639**	1.10 ± 0.6245
EEMP Dose 500 mg/kg bw	20.50 ± 0.5000	60.33 ± 16.2583	0.40 ± 0.1000
EEMP Dose 1000 mg/kg bw	26.83 ± 4.8562	59.00 ± 11.7153	0.63 ± 0.1527
EEMP Dose 2000 mg/kg bw	32.17 ± 4.7521	77.17 ± 4.1932*	0.30 ± 0.1732

Information:
* = There was a significant increase in levels compared to the negative control group (p<0.05)
** = There was a significant increase in levels compared to the negative control group (p<0.01)

(NC) after administration of EEMP test extract for 28 days may indicate that at certain doses, the extract did not have a significant toxic effect on liver and kidney function in female mice of the ddY strain which was used in this study. The statistical data processing process was used the one-way ANOVA method with a post-hoc LSD follow-up test. However, the increase in SGOT levels at doses of 125, 250, and 2000 mg/kg bw although statistically there was a significant difference with the normal control group (p<0.05), but still within the normal range in female mice ddY or BALB/c strains at the age of 1-3 months for AST/SGOT levels, namely at 40-140 U/L (BPOM, 2022; Yoshitake *et al*., 2021). During sub-acute toxicity testing, toxic effects may become more noticeable at the initial dose after administration of the extract. Elevated AST levels may appear faster than changes in other parameters such as ALT or creatinine (Nathwani *et al*., 2005).

CONCLUSIONS

During oral acute toxicity testing for 14 days and oral sub-acute toxicity test for 28 days according to standard methods from BPOM (2022), OECD, and Thompson-Weil in female mice of ddY strain from ethanol extract of liverwort herb *Marchantia paleacea* Bertol. (EEMP) showed a slight increase in biochemical parameters of liver function, namely in SGOT at doses of 125, 250, and 2000 mg/kg bw but variations in SGOT levels in test animals are still normal. But on the entirety of other parameters

did not show any other symptoms of harmful toxicity during acute or sub-acute toxicity testing. In the results of the acute oral toxicity test, there was no death and no signs of preclinical toxicity significantly, therefore, the LD50 value cannot be calculated and can be classified as a mild toxic substance (or has a toxicity level with code 5) based on BPOM criteria (2022). However, more research needs to be done to understand more deeply the potential long-term effects of EEMP as well as to confirm these results in humans clinically.

CONFLICT OF INTEREST

There is no conflict of interest at all in this research grant from the Directorate General of Health Workers of the Ministry of Health of the Republic of Indonesia and UPPM Poltekkes Kemenkes Bandung with Number: DP.04.03/XXV.3.1/760/2023.

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