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The Hepatoprotective Effect of *Marchantia paleacea* Bertol. Extract against Paracetamol-Induced Liver Damage in *Wistar* Rat

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ABSTRACT

Hepatoprotectors are compounds or substances that have efficacy to protect liver cells against toxic substances result in liver damage. Liver damage caused by various factors, such as: viruses, bacteria, drugs that directly damage liver cells (such as paracetamol, etc.), hypersensitivity reactions, hepatotoxic chemicals, toxins in food and pollution. Therefore, the development of the drug is still being tested in order to obtain more satisfactory results in terms of efficacy and the minimal side effects it causes. Until now, there has been no research on the hepatoprotector effect of the liverwort herbs ethanol extract of *Marchantia paleacea* Bertol. in a hepatotoxic-induced test animal model, especially with paracetamol in rats. This study aimed to examine the potential hepatoprotector of the herbal liverworts extract of *Marchantia paleacea* Bertol. compared to Curcuma FCT®. The hepatoprotector effect was determined by examining the biochemical levels of SGPT and SGOT, the percentage of liver index (% w/w) and histopathological feature of the liver. The increase in SGPT and SGOT levels in the liver tissue induced by paracetamol to provoke toxicity of the liver tissue caused by paracetamol. *Marchantia paleacea* Bertol ethanol extract with doses of 26 and 104 mg/kg BW in the measurement of serum levels of SGPT, SGOT, and liver index (% w/w) has shown decreased serum levels of SGPT, SGOT, and liver index (% w/w). In addition, the histological examination for scoring liver cell repair using the *Manja Roenigk Histopathology Scoring Model* method has shown a repair score for paracetamol-induced liver tissue damage at both doses of the test extract (26 and 104 mg/kg BW). Where, the hepatocyte cells tested were generally normal with the characteristics of the nucleus being visible chromatin granules and pink in color with a small number of cells undergoing lysis (karyolysis).

Keywords: *Marchantia paleacea* Bertol., liverwort, paracetamol-hepatotoxicity, liver histology, hepatoprotective

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INTRODUCTION

The liver is one of the most important organs because it has various essential physiological functions and is also the largest organ in the human body, including metabolism of protein, glucose and fat, the process of detoxification, the formation and secretion of various enzymes and bilirubin. The blood supply through the liver reaches 25% of the total blood flow at rest. Hepatic arteries by 30% and portal veins by 70% are the source of blood supply. The speed of blood flow through the liver is needed to compensate for the oxygen demand from the liver cells because more than 50% of its blood supply is from veins. Microorganisms (bacteria, viruses), drugs, toxins and other compounds that are involved in the portal vein will be detoxified in the liver. Because of its complex function, the liver is prone to impaired (Zulkarnain et al., 2017).

Globally, according to WHO data (2018), 788,000 deaths are due to liver cancer and 1.16 million deaths are due to cirrhosis each year, which makes these two types of diseases ranked 11th and 16th most causing deaths each year, respectively. Also, some liver dysfunction accounts for 3.5% of all deaths worldwide. In UK and US, paracetamol (acetaminophen) is the leading cause of drug-induced liver disease. Alcohol and Non-alcoholic fatty liver disease (NAFLD) are the main causes of cirrhosis in Western and other developed countries. Meanwhile, hepatitis B virus is the main cause of cirrhosis in China and other Asian countries (Asrani et al., 2019; *WHO Methods and Data Sources for Country-Level Causes of Death*, 2018). The total prevalence of people with liver dysfunction in Indonesia is based on a study that reports that the prevalence of non-alcoholic fatty liver patients reaches 30% (Amarapurkar et al., 2007).

Paracetamol compounds that are given orally in excess of a certain dose can cause liver damage. Metabolit reaktif yang toksik, yaitu: N-acetyl-p-benzoquinoneimine/NAPQI) dan radikal bebas yang terbentuk melalui proses metabolisme oleh enzim sitokrom P-450 dengan bantuan isoenzim CYP2EI dari senyawa parasetamol/acetaminophen dengan dosis berlebih yang mengakibatkan kerusakan sel hati yang berlebihan. Gangguan integritas membran sel, kerusakan sel hati, dan gagal ginjal dapat terjadi akibat senyawa metabolan radikal bebasnya yang reaktif toksik. Increased levels of the enzymes Glutamate Pyruvate Transaminase/Alanine Amino Transferase (GPT/ALT) and Glutamate Oxaloacetate Transaminase/Aspartate Amino Transaminase (GOT/AST) as a transaminase enzyme become a more specific initial biomarker for detection of liver cell damage (Indahsari et al., 2018).

Hepatoprotector compound agents are an important source of medicine as a complementary/alternative medicine in the management of liver dysfunction because not all malfunctioning diseases can be treated and until now it is still necessary to develop a hepatoprotector agent that is cheap, effective and safe (Adewusi & Afolayan, 2010). The increased antioxidant value of various types and parts of certain plant extracts is often correlated with the efficacy of hepatoprotectors to improve and/or treat liver disease disorders of these parts and plants. (Susilawati et al., 2021). In the form of chemical research, investigations of several genus of liverworts have been carried out which are only 8.8%, which includes: data on testing the activity of antipyretic, anti-inflammatory, treating heart disease, lung disease, antimicrobial, treating various skin diseases, and treating external wounds by the genus of *Bryum*, *Marchantia*, *Sphagnum*, *Octeblepharum*, *Riccia*, *Barbula*, and *Fontinalis*. Bryophytes of the *Marchantia* genus, especially *Marchantia paleaceae* Bertol. is a natural product that has high potential as a source of medicinal produ Several extracts and isolated compounds from Bryophyte have been shown to have antibacterial, antiviral, cytotoxic, nematocidal, insecticidal properties, effects on smooth muscles, weight-loss, plant growth regulators and allelopathic activity (Ceyda İrkin & Tonguc Yayintas, 2018).

One way to deal with or avoid hepatotoxicity is mainly due to the side effect of giving toxic doses of paracetamol, namely by giving hepatoprotector compounds/substances. One plant source that empirically has properties as a hepatoprotector is the herb liverwort *Marchantia paleacea* Bertol. (Fadhilla et al., 2012). This plant contains useful phytochemical compounds, one of which is flavonoid, saponin, phenolic and steroid/triterpenoid compounds. Flavonoid compounds are a class of compounds that are widely known to capture free radicals that cause liver cell damage (Indahsari et al., 2018). Liver cell damage caused by oxidative stress can also be protected by several saponins and terpenoids which are generally antioxidants (Hasballah et al., 2018).

So that researchers are interested in knowing how the potential protection of the ethanol extract of the herb liverworts *Marchantia paleacea* Bertol. as hepatoprotector in male *Wistar* strain rats induced with toxic doses of paracetamol through the determination of biochemical levels of SGPT, SGOT, determination of liver organ index (% w/w) and liver histology examination.

MATERIALS AND METHOD

Materials

The liverwort herb *Marchantia paleacea* Bertol. obtained from Kampung Padajaya, Desa Sindangjaya, Kecamatan Cipanas, Kabupaten Cianjur, West Java and used as plant material in this research. The authenticity of the plant herbs was proven by a botanist at the UPT Kebun Raya Cibodas - LIPI Cianjur with a certificate number: B-0433/IPH.5/AP.0/II/2018. The chemicals used are acetic anhydride (CH_3COOH), iron (III) chloride ($FeCl_3$), magnesium powder, mercury (II) chloride ($HgCl_2$), 1% gelatin, 5% potassium hydroxide (KOH), amyl alcohol, iodine, Liebermann-Burchard reagent, Mayer reagent, Dragendorff reagent, Na-CMC (natrium carboxy methyl cellulose), laboratory standard rat food, aluminum foil, filter paper, SGPT and SGOT assay kit (DiaSys®), hematoxylin-eosin staining solution, Curcuma FCT® was purchased from PT. Kalbe Farma, Tbk., Indonesia. Chloroform ($CHCl_3$), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), amyl alcohol ($C_5H_{12}O$), and ethanol were obtained from PT. Brataco Chemika, West Java, Indonesia.

Experimental Animals

The male *Wistar* rats (*Rattus novergicus*) with the aged 8-12 weeks with an average body weight of 150-200 g was selected in this study and acclimatized for 1 week prior the study. These rats were divided into 6 group of 5 rats.The Health Research Ethics Committee (KEPK) Poltekkes Kemenkes Bandung has approved the method and implementation of this research with an approval number, namely: No. 13/KEPK/EC/XII/2020.

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Methods

Sample extraction

Harvesting, wet sorting, washing, chopping, drying, dry sorting, and maceration extraction processes were carried out with slight modifications to the previous method on liverwort herb samples (Kurniawan et al., 2013; Purkon et al., 2021, 2022).

Marchantia paleacea Bertol. herbs were dried at 50°C for 3 days and blended into a powder, the result was 1600 g of simplicia powder. The herbal simplicia powder sample was extracted with 96% ethanol (w/v) until the simplicia was immersed by the solvent in a macerator and then the maceration extraction process began for 24 hours (25°C) with periodic stirring. The extraction and filtering process was carried out three times. Then, the extract was concentrated at a temperature of 50°C using a rotary evaporator and waterbath so that the yield of thick extract with a fixed weight of 0.97% (w/w) was obtained.

Phytochemical screening

Marchantia paleacea Bertol. extract was evaluated qualitatively with certain reagents for a class of secondary metabolites common in plants. Phytochemical screening is carried out for the

flavonoids, alkaloids, phenolics, tannins, saponins and steroids/triterpenoids (Kurniawan et al., 2013). A positive result indicates a change in color, froth or the formation of a certain precipitate according to the literature.

Pemeriksaan dan evaluasi kadar penanda biokimia gangguan organ hati

The process of examining and evaluating marker compounds in liver function disorders is carried out using the method that has been carried out previously with slight modifications (Novianto & Hartono, 2016; Susilawati et al., 2021). Thirty male *Wistar* rats were weighed and divided randomly into 6 groups. The test dose of *Marchantia paleacea* Bertol. extract given followed previous research (Novianto & Hartono, 2016; Oktavia, S., Ifora, Suhatri dan Susanti, 2017). All feed and drinks were given the same for all treatment groups with only addition of Na-CMC suspending agent in all test groups in the administration.

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Normal (N)	:	No treatment only given 0.5% Na-CMC suspending agent
Negative control (K-)	:	Paracetamol was administered orally (2 g/kg body weight on 8th day)
Positive control (K+)	:	Curcuma FCT® was given orally (81 mg/kg bw) for 7 days and a single dose of paracetamol on 8th day (Novianto et al., 2016)
Dose I (D1)	:	*Marchantia paleacea* Bertol. extract given orally (26 mg/kg bw) for 7 days and a single dose of paracetamol on 8th day
Dose II (D2)	:	*Marchantia paleacea* Bertol. extract given orally (52 mg/kg bw) for 7 days and a single dose of paracetamol on 8th day
Dose III (D3)	:	*Marchantia paleacea* Bertol. extract given orally (104 mg/kg bw) for 7 days and a single dose of paracetamol on 8th day

At the 8th day after the administration of last dose, the rats were anesthetized systemically (tends to be euthanized) using carbon dioxide gas (CO_2), surgery on the area (thorax) and taking blood from the heart using a 3 mL syringe. The heartbeat after the euthanasia process still works for approximately 3 minutes so that surgery and intracardiac blood collection can be performed (Adyttia et al., n.d.). The blood that has been drawn is collected in eppendorf then centrifuged at 3000 rpm for 10 minutes and then the serum is separated for analysis for serum levels of GPT and GOT (Sebayang et al., 2020). By using 0.9% NaCl solution (saline), the liver that has been taken is taken for repair and dried immediately evenly. Then the cleaned liver, stored, and fixed in neutral buffered formalin (NBF) 4% with a pH of 7.4. In the examination of liver index (% w/w), rat liver was cleaned with physiologic NaCl (saline) 0.9%, dried with a thick tissue, examined, and weighed macroscopically. Liver index was calculated as liver weight divided by rat body weight (% w/w) (Freitag et al., 2015).

100 μL of serum was put into a 1.5 mL vacutainer tube (eppendorf), then 1000 μL of SGPT (ALT) determining reagent (DiaSys®) was added. The resulting mixture of serum and working reagent SGPT (ALT) was incubated at 20-25℃ for 1 minute, then the absorption was read using a clinical UV photometer (KENZA MAX BioChemisTry – Biolabo Diagnostics®) at a wavelength of 340 nm. In determining the levels of SGOT/AST (DiaSys®) all test serum was carried out with the same amount of serum and working reagent as SGPT (ALT), including the examination temperature (20-25℃), and incubation time (1 minute). The levels of SGPT (ALT) and SGOT (AST) were obtained by the formula below:

$$\text{SGPT and/or SGOT (U/L)} = \frac{\Delta A/\min Sample}{\Delta A/\min Calibrator/Standard} \text{ x Conc. Calibrator (U/L)}$$

Liver index and evaluation of histological examination

Histopathological studies of the liver have been carried out based on the previous method (Hasballah et al., 2018; Ilyas et al., 2019). Buffered neutral formaline was used for the fixation of liver tissue with small pieces which were then carried out for histopathological examination and other tissue imaging processes. Staining with hematoxylin and eosin solutions was carried out on five micron sections taken and then assessed for any changes in the histological structure using a light microscope. The scoring method using the *Manja Roenigk Histopathology* scoring method was carried out qualitatively and quantitatively on the condition of hepatocytes and blood vessels. In the process of reading the number of normal cells, parenchymal degenerated cells, hydropic degenerated cells, and necrotic cells were carried out using a microscope with a magnification of 400 times in five fields of view which each field of view had 20 cells. So that in 1 preparation can be read as many as 100 cells. The average amount of each is sought to be multiplied by the *Manja Roenigk Histopathology* score. The results of these scores are summarized and the *Manja Roenigk Histopathology* damage score is obtained of one rat (Hasballah et al., 2018).

Data Analysis

The total levels of biochemical markers of SGPT and SGOT and the level of hepatocyte damage were analyzed using the SPSS software program. Values are expressed in Mean ± Standard error of mean (SEM). The difference in the data of each test group was tested statistically to see the level of difference in significance with the One-way Anova method on the SPSS 25 application. Then proceed to compare the data of each group against the negative control group with the LSD Post-hoc test. The data of each test group that has been compared to the control group can be said to have a significant difference if it is more than $P<0.05$ statistically.

RESULT AND DISCUSSION

Phytochemical screening

Screening examination for phytochemical content of natural ingredients as a therapeutic agent or its pharmacological activity cannot be separated. In the process of screening secondary metabolites from plant extracts, this technique can be used (Handayani et al., 2017; Purniawan et al., 2013). In this study, a qualitative screening test was carried out and the results are presented in Table 1.

Table 1. Phytochemical content contained in *Marchantia paleacea* Bertol. herb ethanol extracts

Compounds	Result	
	***Marchantia paleacea* Bertol. ethanol extract**	**Observation**
Flavonoid	(+)	An orange/yellow ring was formed
Saponin	(+)	The foam is stable for not less than 10 minutes, 2-5 cm high
Phenolic	(+)	Blackish blue
Tanin	(+)	White precipitate
Steroid/Triterpenoid	(+)	Purplish green/purple

+ = Present, - = Absent

Marchantia paleacea Bertol. herbs contain various bioactive phytochemical compounds. Table 1 shows the presence of flavonoids, saponins, phenolics, tannins and steroids/triterpenoids. Previously, that Asakawa et al. (2009) and Xiao et al. (2006) found that the *Marchantia* genus contained terpenoid compounds (monoterpenoids, sesquiterpenoids, diterpenoids and triterpenoids) and simple phenolic compounds. Steroid compounds, flavonoids and bibenzyls are also found in *Marchantia paleacea* Bertol. (Wang et al., 2013). Previous findings indicate that phytochemicals of

the genus *Marchantia* (liverworts) have potential effects as antimicrobials (Fadhilla et al., 2012), anti-inflammatory, antipyretic, antitoxin, antiseptic and diuretic (Asakawa, 2008).

Biochemical results

Measurement of biochemical markers of liver function was carried out to evaluate the levels of SGPT and SGOT from rats with different treatments. In determining liver toxicity, SGPT and SGOT are used as markers of liver toxicity because they are mainly found in the liver (Novianto & Hartono, 2016). The results of the activity of SGPT and SGOT are presented in Table 2.

Table 2. The serum glutamic pyruvic transaminase (SGPT) enzymes activity in different treatments on paracetamol-induced rats

Treatments	Biochemical SGPT Parameter (U/L)		
	Early (1st day)	After Giving the Test Extract (7th day)	After Induction (9th day)
Normal (N)	79.45 ± 1.620	62.60 ± 2.209	62.47 ± 1.997[c]
Negative control (K-)	70.20 ± 1.691	68.45 ± 0.865	134.03 ± 12.052
Positive control (K+)	59.63 ± 1.600	55.15 ± 1.074[b]	88.60 ± 4.777[a]
Dose I (D1)	75.23 ± 2.005	58.13 ± 2.144[a]	76.70 ± 5.629[b]
Dose II (D2)	78.78 ± 2.161	52.40 ± 1.627[c]	63.47 ± 5.773[c]
Dose III (D3)	55.08 ± 1.915[b]	37.78 ± 1.171[c]	53.30 ± 2.039[c]

*Note: Data are expressed as mean ± SEM. The mean data with letters written in superscript was significantly different (a = P <0.1; b = P <0.05; c = P <0.01) with the negative control group.

Table 2 and Table 3 show that the treatment of the negative control group/K- (paracetamol) had the same significant increase in SGPT and SGOT levels was 134.03 ± 12.052 U/L (P<0.01) and 255.27 ± 12.117 U/L (P<0.01) compared with the normal treatment 62.47 ± 1.997 U/L (SGPT) and 107.60 ± 1.998 U/L (SGOT). The three test groups of the test ethanol extract (*Marchantia paleacea* Bertol.) can reduce serum activity for SGPT and SGOT, but the lowest significant decrease in levels was shown by the BW group of 104 mg/kg (dose 3/D3) with an average final SGPT and SGOT levels. 53.30 ± 2.039 U/L (P<0.01) and 160.30 ± 6.178 U/L (P<0.05) respectively, which were almost the same or close to normal and the D3 treatment group. Even though the initial SGPT levels from group D3 were the lowest initial levels, it could be seen in the final results (9th day) that they still showed SGPT levels that were higher/closer to the initial levels compared to the other two test extracts. Meanwhile, the three test extracts were similar in producing a decrease that was closer to/better to the usual dose than the positive control group (K+) of the positive control group (K+) Curcuma FCT® (81 mg/kg bw). The decrease in final SGPT and SGOT levels from the comparison group Curcuma FCT®, respectively, were: 18[illegible]0 ± 4.777 U/L and 190.63 ± 17.846 U/L. The final serum liver enzyme activity also decreased in the treatment group at dose 1 (26 mg/kg bw) and dose 2 (52 mg/kg bw), namely dose 1 [(SGPT: 76.70 ± 5.629 (P<0.05) and SGOT : 179.50 ± 3.829 (P<0.1)] and dose 2 [SGPT: 63.47 ± 5.773 (P<0.01) and SGOT: 184.10 ± 12.217 (P<0.1)] were compared with negative control (K-).

Table 3. The serum glutamic oxaloacetic transaminase (SGOT) enzymes activity in different treatments on paracetamol-induced rats

Treatments	Biochemical SGOT Parameter (U/L)		
	Early (1st day)	After Giving the Test Extract (7th day)	After Induction (9th day)
Normal (N)	103.78 ± 9.840	112.85 ± 5.651	107.60 ± 1.998[c]

Negative control (K-)	130.35 ± 6.670	133.88 ± 2.943	255.27 ± 12.117
Positive control (K+)	99.20 ± 2.033[a]	112.18 ± 7.858	190.63 ± 17.846[a]
Dose I (D1)	109.25 ± 2.206	126.93 ± 2.695	179.50 ± 3.829[a]
Dose II (D2)	102.53 ± 2.211	90.85 ± 1.740[b]	184.10 ± 12.217[a]
Dose III (D3)	89.95 ± 1.470[b]	99.55 ± 2.033[b]	160.30 ± 6.178[b]

*Note : Data are expressed as mean ± SEM. The mean data with letters written in superscript was significantly different (a = P <0.1; b = P <0.05; c = P <0.01) with the negative control group.

Increased levels of SGPT and SGOT indicate paracetamol-induced liver tissue toxicity. This finding is in line with Olaleye et al. (2010) who reported that one of the methods of detecting liver damage or injury can use the determination of specific biochemical parameters, such as: SGPT and SGOT. An increase in SGPT levels two times the level usually indicates liver tissue damage (Olaleye et al., 2010). Seif et al. (2016) also reported that paracetamol causes an increase in the index of serum liver function such as SGPT and SGOT, causing free radical formation, fat peroxidation, mitochondrial dysfunction, hepatocellular damage, decreased liver function and impaired natural immune system (innate immunity), glutathione deficiency which leads to hepatotoxicity (Seif, 2016). The administration of the three test extracts could reduce SGPT and SGOT levels in the group of rats with hepatotoxicity with the best reduction in levels at a dose of 104 mg/kg bw. In other words, decreased levels of SGPT and SGOT in rats with hepatotoxicity could be reduced by using ethanol extract of the liverworts of *Marchantia paleacea* Bertol.

Liver index and histopathological results

The efficacy of all test groups on the liver weight of rats with paracetamol-induced hepatotoxicity is presented in Table 4. A significant difference (P<0.01) in the liver weight observed after experiencing paracetamol-induced hepatotoxicity (K-) was 4.13 ± 0.01 % w/w against the normal group (N) of 3.55 ± 0.04 % w/w. The liver index increased in the negative control group (K-), but returned to normal values in the test extract group and positive control (K+).

The dose 1 (D1) test extract 26 mg/kg BW had a very significant reduction (P<0,01) which is 3.51 ± 0.03 % w/w compared to the negative control (K-) in the liver index. This decrease also occurred in the two other test extracts (52 and 104 mg/kg BW) and also the Curcuma FCT® comparison group which both had no significant reduction (P <0.1) against negative controls (Table 4). The condition of decreasing levels of SGPT and SGOT as recovery/repair of liver cells in experimental rat treated according to previous studies (Chaphalkar et al., 2017).

Table 4. Liver index percentage (% w/w)

Treatments	Average		
	Test Animal Body Weight of Rats (g)	Liver Weight (g)	Percentage of Liver Index (% w/w)
Normal (N)	173.00 ± 7.85	6.171 ± 0.346	3.55 ± 0.04[c]
Negative control (K-)	179.33 ± 3.70	7.410 ± 0.158	4.13 ± 0.01
Positive control (K+)	189.67 ± 4.83	7.694 ± 0.312	4.04 ± 0.07[a]
Dose I (D1)	173.67 ± 2.00	6.101 ± 0.097	3.51 ± 0.03[c]
Dose II (D2)	162.67 ± 3.99	6.196 ± 0.141	3.82 ± 0.07[a]
Dose III (D3)	170.67 ± 2.48	6.761 ± 0.171	3.96 ± 0.05[a]

*Note: Data are expressed as mean ± SEM. The mean data with letters written in superscript was significantly different (a = P <0.1; b = P <0.05; c = P <0.01) with the negative control group.

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Histopathological grade measures for liver cells were assessed by *the Manja Roenigk Histopathology* scoring technique. In this method of reading the histopathological examination scoring method uses five different views on liver tissue preparations. In each field there are 20 cells so that in each (one) preparation 100 cells can be read with the data used in the form of an average ± SEM (with a magnification of 400x). Each observation is then multiplied by the score of each cell. Normal scores were given a score scale of 1, cells with parenchymal degeneration on a score of 2, cells with hydropic degeneration were given a score of 3, and cells with necrosis were given a score of 4 as described in previous studies (Hasballah et al., 2018; Ilyas et al., 2019; Sabri et al., 2018). The scores for each treatment are as shown in Table 5 and Figure 1.

Table 5. Mean and histopathological value of rat liver in the data of liver cell damage rate in *the Manja Roenigk Histopathology* scoring method of all test groups

Treatments	N	Assess the Level of Liver Cell Damage (Mean ± SEM)
Normal (N)	5	1.134 ± 0.063^{c}
Negative control (K-)	5	3.062 ± 0.152
Positive control (K+)	5	1.462 ± 0.083^{c}
Dose I (D1)	5	2.302 ± 0.186^{c}
Dose II (D2)	5	2.758 ± 0.134
Dose III (D3)	5	1.408 ± 0.122^{c}
Total	30	2.021 ± 0.144

*Note: Data are expressed as mean ± SEM. The mean data with letters written in superscript was significantly different (a = P <0.1; b = P <0.05; c = P <0.01) with the negative control group.

Various forms of cell categories were observed including normal cells, parenchymal degenerated cells, hydropic degenerated cells, and necrotic cells. Swelling and cloudiness in cells produced by the appearance of granules in the cytoplasm or protein which is the mildest type of degeneration, parenchymal degeneration. Disruption of oxidative reactions in mitochondria and endoplasmic reticulum causes a reversible type of degeneration. The affected cells cannot eliminate the water so that the cells swell (Luedde et al., 2014). In the event of hydropic degeneration, the cytoplasm containing vacuoles only contains water and does not contain fat and glycogen so that the cytoplasm swells and looks pale due to an increase in fluid. So that hydropic degeneration is a more severe type of degeneration. Hypoxia or even chemical poisoning is a type of metabolic disorder symptom that indicates hydropic degeneration. This type of degeneration is curable unless the underlying cause is cancer. Cell death in this type of degeneration can be caused by changes in the cell nucleus that damage the plasma membrane in injured cells, resulting in cell death. Necrosis is a pathological process of cells if they have been injured. Changes in the shape of the cytoplasm and the nucleus of the cell indicate the occurrence of necrosis. The increase in the color of eosin due to the occurrence of eosin and protein bonds in the cytoplasm indicates that the cell is undergoing necrosis (Hasballah et al., 2018). The photomicrograph of each different treatment group can be seen in figure 1.

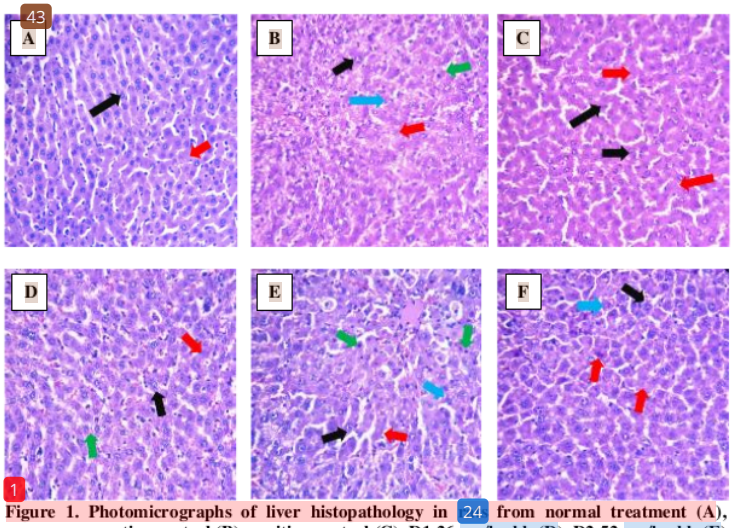

Figure 1. Photomicrographs of liver histopathology in from normal treatment (A), negative control (B), positive control (C), D1 26 mg/kg bb (D), D2 52 mg/kg bb (E) and D3 104 mg/kg bb group (F) with 400x magnification. Black arrows: indicate generally normal cells with characteristic cell nuclei with visible chromatin granules and pink cytoplasm; red arrow: indicates that there are a few cells that die in the form of karyolysis, where cells do not have a nucleus; arrows in blue: indicate the cell has hydrophilic degeneration; green arrow: indicates cells undergoing necrosis with compacted cell nucleus.

Figure 1 shows the photomicrograph of each different treatment group. The normal group (Figure 1A) showed normal hepatocytes with chromatin granules and cytoplasm in pink (black mark), no inflammatory infiltration and only a few cells that experienced cell death in the form of karyolysis where the cells did not have a nucleus (red arrow). In the negative control group/K- (paracetamol) in Figure 1B, cells appear to be generally damaged with various degrees ranging from hydrophic degeneration (blue arrow), necrosis with a condensed nucleus (green arrow) and missing nuclei (red arrow) even though there are visible cells normal (black arrow). Positive control group (K+) in Figure 1C and *Marchantia paleacea* Bertol. extract at a dose of D1 26 mg/kg bw (Figure 1D) and a dose of D3 104 mg/kg bw (Figure 1F) showed the similarity of liver tissue with the normal group experiencing a little infiltration of inflammatory cells whose cells were generally normal with the characteristics of nucleus showing chromatin granules and cytoplasm in pink (black arrow). However, there are a few cells that experience damage in the form of hydrophic degeneration (blue arrow) and death in the form of karyolysis where cells do not have a nucleus (red arrow). Treatment of *Marchantia paleacea* Bertol. extract at doses of D1 (26 mg/kg bw) and D2 (104 mg/kg bw) in rats induced by paracetamol also showed enhancement in hepar tissue. Liver tissue repair was observed from the dominance of normal hepatocytes compared to cell lysis (karyolysis) and liver tissue repair.

Paracetamol administration damage liver tissue and result in histological changes of liver cells. Paracetamol accelerates the production of free radicals such as the formation of toxic reactive

metabolites (N-acetyl-p-benzoquinoneimine/NAPQI) and free radicals through the biotransformation process by cytochrome P-450 enzymes with the help of CYP2EI isoenzymes (Indahsari et al., 2018). Large lipid peroxidation in liver tissue can disrupt liver cells and form parenchymal necrosis in hepatocyte cells due to the use of toxic (large) doses of paracetamol. Free radicals that are formed such as: alkoxyl, aldehyde, and peroxyl from lipid peroxidation reactions can cause damage to liver cells and release certain marker enzymes such as SGPT and SGOT (Abou Seif, 2016).

A major step in the pathogenesis of paracetamol toxicity is inflammation caused by infiltration of endogenous leukocytes (such as: Kuppfer cells, macrophages, and neutrophils). Several studies have reported on lipid peroxidation in the heart and liver by reactive oxygen species causing tissue damage by paracetamol (Jaeschke & Ramachandran, 2020; Lawson et al., 2000). Polyunsaturated fatty acids in membrane lipids, proteins, genetic material, and hepatocytes are attacked by ROS which damage the hepar. Antioxidants are involved in dealing with free radicals and inhibit cell damage by the process of lipid peroxidation (Jaeschke & Ramachandran, 2020). The findings of this study indicate a decrease in the levels of biomarkers of liver damage and liver tissue repair with *Marchantia paleacae* Bertol. extract at various concentrations in rats induced hepatotoxicity with paracetamol which is still associated with previous research on *Marchantia* genus as an antioxidant against various free radicals (Gökbulut et al., 2012; Sinam et al., 2016).

CONCLUSION

Marchantia paleacea Bertol ethanol extract. D1 (26 mg/kg BW) and D3 (104 mg/kg BW) showed a significant reduction in SGPT and SGOT levels and liver tissue improvement in animal model induced by hepatotoxicity with paracetamol. These findings suggest a potential protective effect on liver function against paracetamol-induced hepatotoxicity in rat liver. thus, the herb liverwort *Marchantia paleacea* Bertol has great potential as a nutraceutical product, supplement, or traditional medicine that has hepatoprotective therapeutic efficacy.

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