Journal of Research in Pharmacy

Research Article

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The hepatoprotective effect of *Marchantia paleacea* bertol. extract against acetaminophen-induced liver damage in rat: biochemical and histological evidence

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Received: 22 March 2022 / Revised: 30 June 2022 / Accepted: 01 July 2022

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 acetaminophen, etc.), hypersensitivity reactions, hepatotoxic chemicals, toxins in food and pollution. Therefore, the development of the drug is still being tested 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 acetaminophen in rats. This study aimed to examine the potential hepatoprotector of the herbal liverworts extract of *Marchantia paleacea* Bertol. compare 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 acetaminophen to prove the toxicity of the liver tissue caused by acetaminophen. *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 acetaminophen-induced liver tissue damage at both doses of the test extract (26 and 104 mg/kg bw). These findings suggest that 26 and 104 mg/kg bw have a potential protective effect on liver function against hepatotoxicity in rat livers. Thus, *Marchantia paleacea* Bertol. liverwort herb has great potential as a nutraceutical product, supplement, or traditional medicine that has hepatoprotective therapeutic efficacy.

KEYWORDS: *Marchantia paleacea* Bertol.; acetaminophen-induced; hepatoprotective; SGPT and SGOT; liver histology.

1. INTRODUCTION

Liver is the largest organ in the body and has various important functions, 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. Sources of blood supply are the hepatic artery (30%) and portal vein (70%). 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

How to cite this article: Purkon DB, Kusmiyati M, Trinovani E, Fadhlillah FM, Widyastiwi W, Roseno MH, Khristian E, Nadhifah A. The hepatoprotective effect of *Marchantia paleacea* bertol. extract against acetaminophen-induced liver damage in rat: biochemical and histological evidence. J Res Pharm. 2022; 26(6): 1857-1867.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.275

the portal vein will be detoxified in the liver. Because of its complex function, the liver is prone to impaired [1,2].

Globally, according to WHO data (2018), cirrhosis currently causes 1.16 million deaths and liver cancer causes 788,000 deaths, 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 the UK and US, acetaminophen (paracetamol) is the leading cause of drug-induced liver disease. The main causes of cirrhosis in Western and other developed countries are alcohol and NAFLD (non-alcoholic fatty liver disease), while the main cause of cirrhosis in China and other Asian countries is the hepatitis B virus [3,4]. 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% [5,6].

Acetaminophen compounds that are given orally more than a certain dose can cause liver damage. Excessive damage to liver cells by acetaminophen is caused by 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. Toxic reactive metabolites and free radicals can disrupt the integrity of cell membranes, leading to liver cell damage and kidney failure. 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. One way to deal with or avoid hepatotoxicity is mainly due to the side effect of giving toxic doses of acetaminophen, namely by giving hepatoprotector compounds/substances [7,8].

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 [9]. Many research results from plant extracts have a hepatoprotector effect by increasing their antioxidant value so that types of medicinal herbs (plants) play an important role in treating liver diseases or disorders [10]. Only about 8.8 % of liverworts have been chemically investigated, which types such as *Bryum*, *Marchantia*, *Sphagnum, Octeblepharum*, *Riccia*, *Barbula* and *Fontinalis* have been tested for different diseases such as heart disease, fever, inflammation, lung disease, various infections, skin diseases and external wounds. Bryophytes of the *Marchantia* genus, especially *Marchantia paleaceae* Bertol. is a natural product that has high potential as a source of medicinal products. Several extracts and isolated compounds from Bryophyte have been shown to have antimicrobial, antiviral, cytotoxic, nematocidal, insecticidal properties, effects on smooth muscles, weight loss, plant growth regulators and allelopathic activity [11]. One plant source that empirically has properties as a hepatoprotector is the herb liverwort *Marchantia paleacea* Bertol. [12]. Actually, there are other species of the genus *Marchantia* that have been scientifically tested for hepatoprotector activity, namely testing the activity of hepatoprotectors from flavonoid compounds obtained from *Marchantia polymorpha* in rat test animal models induced hepatotoxic by CCl_4 compounds [13]. And also testing anti-hepatitis activity of flavonoid compounds obtained from *Marchantia convoluta* [14]. From the two tests of the hepatoprotector with 2 different species of the genus *Marchantia* showed a decrease in the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), physiological biochemical indicators of other liver organs, and repaired cell damage in the liver by histological examination. But research on the activity of hepatoprotector of the plant species *Marchantia paleacea* Bertol. has never been done.

This plant contains useful phytochemical compounds such as flavonoids, saponins, phenols, and steroids/triterpenoids. Flavonoids are a class of compounds commonly known to scavenge free radicals that cause liver cell damage [7]. Saponins and terpenoids are also antioxidants that can protect the liver from oxidative stress [15]. Therefore, researchers are interested in determining the potential protection of the ethanol extract of the liverwort *Marchantia paleacea* Bertol. as a hepatoprotectant in male Wistar rats treated with toxic doses of acetaminophen by determining the biochemical levels of SGPT, SGOT, liver organ index (% w/w), and histological examination of the liver.

2. RESULTS AND DISCUSSION

2.1 Phytochemical screening

Screening examination for phytochemical content of natural ingredients as a therapeutic agent or its pharmacological activity cannot be separated. This technique is used to analyze secondary metabolites found

in plant extracts [16,17]. 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. (2008) and Xiao et al. (2005) 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. [18]. Previous findings indicate that phytochemicals of the genus *Marchantia* (liverworts) have potential effects as antimicrobials [12], anti-inflammatory, antipyretic, antitoxin, antiseptic and diuretic [19, 20].

2.2 Biochemical results

Measurement of biochemical markers of liver function was carried out to evaluate the levels of SGPT and SGOT from rats with various treatments. In identifying liver toxicity, SGPT and SGOT are used as markers of liver toxicity because they are mainly found in the liver [21]. 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 acetaminophen-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^{cRe}

*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.

Curcuma FCT® is a traditional medicine of the "jamu" group produced by PT. SOHO Global Health, Tbk. with distribution permit number TR052549901 by the Food and Drug Supervisory Agency/BPOM of the Republic of Indonesia in the form of a membrane-coated tablet available which is marketed in Indonesia and several other countries. Each curcuma FCT® tablet preparation contains 20 mg of *Curcuma xanthorrhiza* Roxb extract which contains curcumin compounds and can improve liver organ function, treat jaundice, and improve appetite [22, 23]. The drug compound has been widely used as a comparison drug (positive control) in testing the activity of other hepatoprotectors as tested by Hariyanti, L. P. D., et al. (2017); SR, Muhammad Asri, et al. (2021); and Suriani, S., et al. (2013). Curcumin compounds from *Curcuma xanthorrhiza* Roxb extract has a mechanism of action of one of the hepatoprotectors by capturing and breaking between superoxide ions (O^{2-}) so as to prevent damage to liver cells. In addition, curcumin compounds may also increase glutathione S-transferase (GST) which has the activity of inhibiting several proinflammatory factors. The dose used in the suspension preparation of the drug Curcuma FCT was 81 mg/kg bw orally [22–25].

Table 2 and Table 3 show that the treatment of the negative control group/K- (acetaminophen) 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 dose 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 results (9th day) that they still showed SGPT levels that were higher/closer to the initial levels compared to the other two test extracts. In this SGPT level examination, it was seen the effect of the amount of dose given in each group on the improvement of the decrease in SGPT levels. The greater the dose administered (D3), the more significantly the SGPT levels of the negative control group (P<0.01). 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: 88.60 ± 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 acetaminophen-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 acetaminophen-induced liver tissue toxicity. Selection of acetaminophen doses that can induce hepatotoxic events based on some scientific literature of similar hepatoprotector studies in rat test animals with minor modifications [26–28]. This finding is in line with Olaleye et al. (2010) who reported that SGPT and SGOT are specific biochemical parameters for detecting liver damage or injury. An increase in SGPT levels two times the level usually indicates liver tissue damage [28]. Seif et al. (2016) also reported that acetaminophen 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 [29]. 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, the ethanol extract of the liverwort herb *Marchantia paleacea* Bertol. has the effect of reducing SGPT and SGOT levels in rats experiencing hepatotoxicity.

2.3 Liver index and histopathological results

The efficacy of all test groups on the liver weight of rats with acetaminophen-induced hepatotoxicity is presented in Table 4. A significant difference (P<0.01) in the liver weight observed after experiencing acetaminophen-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 the Curcuma FCT® comparison group which both had no significant reduction (P <0.1) against negative controls (Table 4). This finding is in line with previous studies where a decrease in SGPT and SGOT levels led to the recovery of liver cells in treated rats [30].

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.

Histopathological grade criteria for liver cells were assessed by *the Manja Roenigk Histopathology* scoring method. The scoring method used was to read each liver tissue preparation in five different views (in one field of view there are 20 cells so that in 1 preparation 100 cells are read) and counted the mean ± SEM of cells of the preparation with a magnification of 400x. Then each discussion is multiplied by the score of each cell. The score for each cell which includes normal cells (score 1), parenchymatous degeneration (score 2), hydropic degeneration (score 3) and necrosis (score 4) as shown in previous studies [15,31,32]. 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.

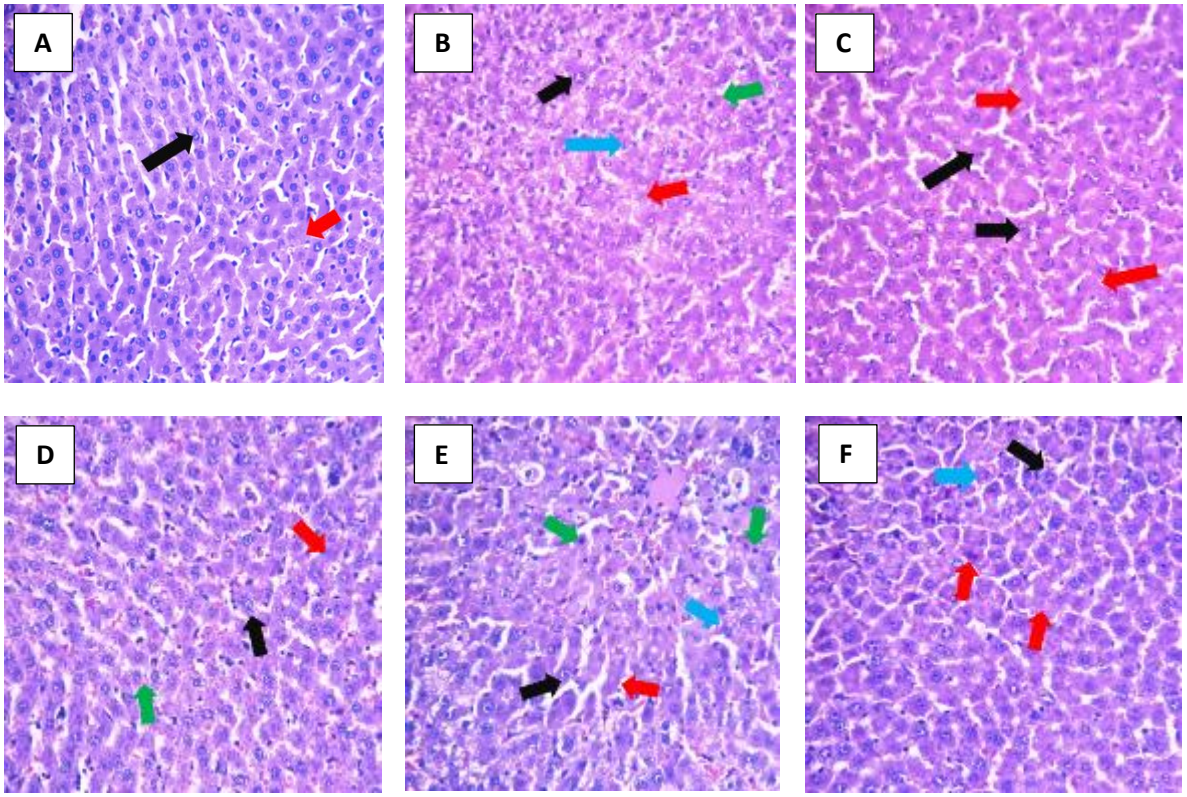

Figure 1. Photomicrographs of liver histopathology in rats from normal treatment (A), negative control (B), positive control (C), D1 26 mg/kg bb (D), D2 52 mg/kg bb (E) and D3 104 mg/kg bb group (F) with 400x magnification. Black arrows: indicate generally normal cells with characteristic cell nuclei 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.

The classification of cell damage is divided into normal cells, parenchymal degeneration, hydropic degeneration, and necrosis. Parenchymal degeneration is the mildest degeneration, due to swelling and cloudiness of the cytoplasm due to the appearance of granules in the cytoplasm due to protein deposition. This degeneration is reversible because it only occurs in the mitochondria and endoplasmic reticulum due to interference with oxidative reactions. The affected cells cannot eliminate the water so that the cells swell [33]. Hydropic degeneration is degeneration that is more severe. In this degeneration, water containing vacuoles in the cytoplasm does not contain fat or glycogen, so that the cytoplasm becomes pale and swollen with buildup of fluid. Hydropic degeneration usually occurs due to metabolic disorders such as hypoxia or chemical poisoning. This degeneration is also curable, but it may be irreversible if there is a cancer cause. The injured cell will experience damage in the plasma membrane and a change in the cell nucleus will occur which will result in cell death. Necrosis is a pathological process of cells if they have been injured. Necrosis is characterized by changes in the cytoplasm and cell nucleus. Changes in the cytoplasm of necrotic cells will show an increase in eosin color due to increased bonds between eosin and cytoplasmic proteins [15]. The photomicrograph of each different treatment group can be seen in Figure 1.

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- (acetaminophen) 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

acetaminophen also showed improvement in liver tissue. Liver tissue repair was observed from the dominance of normal hepatocytes compared to cell lysis (karyolysis) and liver tissue repair.

Administration of acetaminophen (paracetamol) damages liver tissue and causes histological changes in liver cells. Acetaminophen 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 isozymes [7]. Acetaminophen leads to severe lipid peroxidation in liver tissue in the form of hepatocyte formation and parenchymal necrosis. Lipid peroxidation produces free radicals such as alkoxyl, aldehyde, and peroxyl, which lead to cell damage and the release of marker enzymes (Abou Seif, 2016).

A significant step in the pathogenesis of acetaminophen 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 acetaminophen (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 acetaminophen which is still associated with previous research on *Marchantia* genus as an antioxidant against various free radicals [34,35].

3. CONCLUSION

Marchantia paleacea Bertol ethanol extract. D1 (26 mg/kg body weight) and D3 (104 mg/kg body weight) showed significant reduction in SGPT and SGOT levels and improvement in liver tissue in an animal model induced by hepatotoxicity with acetaminophen. These results suggest a potential protective effect on liver function against acetaminophen-induced hepatotoxicity in rat liver. Thus, the liverwort Marchantia paleacea Bertol has great potential as a nutraceutical product, dietary supplement, or traditional medicine with hepatoprotective therapeutic activity.

4. MATERIALS AND METHODS

4.1. 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, iron (III) chloride, magnesium powder, mercury (II) chloride, 1% gelatin, 5% potassium hydroxide, 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. SOHO Global Health, Tbk., Indonesia. Chloroform, hydrochloric acid, sulfuric acid, amyl alcohol, and ethanol were obtained from PT. Brataco Chemika, West Java, Indonesia.

4.2. Experimental animals

Thirty-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 test animals were fed normally with standard feed of rat test animals laboratory. These rats were divided into 6 group of 5 rats [36]. This research has been passed as ethical approval by the Health Research Ethics Committee (KEPK) of Poltekkes Kemenkes Bandung with ethical approval number: No. 13/KEPK/EC/XII/2020.

4.3. Methods

4.3.1. Sample extraction

Harvesting, wet sorting, washing, chopping, drying, dry sorting and maceration extraction processes on liverwort herb samples were determined according to the previous method including some modifications [17].

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 extract filtrate was filtered, and the extraction process was repeated 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.

4.3.2 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 [17]. A positive result indicates a change in color, froth or the formation of a certain precipitate according to the literature.

4.3.3 Evaluation of hepatic biochemical markers

The evaluation of biochemical markers of liver function followed the previous method with modifications [6,10,21,37]. Thirty male *Wistar* rats were weighed and divided randomly into 6 groups. The test dose of *Marchantia paleacea* Bertol. extract given followed previous research [21,38]. 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.

Normal (N)	:	No treatment only given 0.5% Na-CMC suspending agent
Negative control (K-)	:	Acetaminophen 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 acetaminophen on 8th day [2,6,10]
Dose I (D1)	:	*Marchantia paleacea* Bertol. extract given orally (26 mg/kg bw) for 7 days and a single dose of acetaminophen on 8th day
Dose II (D2)	:	*Marchantia paleacea* Bertol. extract given orally (52 mg/kg bw) for 7 days and a single dose of acetaminophen on 8th day
Dose III (D3)	:	*Marchantia paleacea* Bertol. extract given orally (104 mg/kg bw) for 7 days and a single dose of acetaminophen on 8th day

At the 9th day after fasting for approximately 18 hours after administration of acetaminophen as a hepatotoxic inducer (administration of acetaminophen on the 8th day, while the last administration of the extract test and positive control extract preparation was given on the 7th day), 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 [39]. 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 [40]. The enzymatic activity of serum Glutamic Pyruvate Transaminase (GPT) and Glutamic Oxaloacetate Transminase (GOT) was measured according to the previous method [41,42]. The liver was immediately removed and cleaned with 0.9% NaCl (saline). 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 physiological 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) [27].

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°C 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°C), and incubation time (1 minute). The levels of SGPT (ALT) and SGOT (AST) were obtained by the formula below:

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

.3.4. Liver index and evaluation of histological examination

Histopathological studies of the liver have been carried out based on the previous method [15,32]. Small pieces of liver tissue were fixed with 4% neutral buffered formalin for histopathology and routine tissue processing. Five-micron pieces were taken, stained with Hematoxylin & Eosin staining solution, and assessed for any changes in the histological structure under light microscopy. The analysis was carried out qualitatively and quantitatively with the *Manja Roenigk Histopathology* scoring method by observing the condition of the hepatocytes and blood vessels. Each preparation was observed using a microscope with a magnification of 400 times in five fields of view (in one field of view there are 20 cells so that in 1 preparation 100 cells are read) and counted the number of normal cells, the number of cells experiencing parenchymal degeneration, hydropic degeneration, and necrosis. 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 [15].

4.4 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 form of analysis testing includes testing the distribution of data normality, homogeneity, and One-way ANOVA was performed to see the significance of differences between groups. Then proceed to compare the data of each group against the negative control group with the LSD Post-hoc test. $p<0.05$ was considered statistically significant.

Acknowledgements: The author would like to acknowledge UPPM Poltekkes Kemenkes Bandung and Directorate General of Health Workers (Dirjen Nakes) for funding support in this research and UPT KR. Cibodas-LIPI, Cianjur for support and provision of facilities.

Author contributions: Concept – D.B.P., F.M.F.; Design – D.B.P., W.W., M.H.R.; Supervision – M.K., E.T.; Resources – D.B.P., E.K., A.N.; Materials – D.B.P., E.K., A.N.; Data Collection and/or Processing – D.B.P., E.K., A.N.; Analysis and/or Interpretation – F.M.F., D.B.P.; Literature Search – D.B.P., F.M.F., W.W., M.H.R., E.K.; Writing – D.B.P., F.M.F.; Critical Reviews – M.K., E.T., E.K.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

[1] Zulkarnain Z, Novianto F, Saryanto S. Phase II clinical trial of jamu for hepatoprotector "(article in Indonesian with an abstract in English)". Buletin Penelitian Kesehatan. 2017;45:125–136. [CrossRef]

[2] Yuvaraja KR, Santhiagu A, Jasemine S, Gopalasatheeskumar K. Hepatoprotective activity of *Ehretia microphylla* on paracetamol induced liver toxic rats. Marmara Pharm J. 2021;25(1):89–98. [CrossRef]

[3] Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol. 2019;70(1):151–171. [CrossRef]

[4] WHO, WHO methods and data sources for country-level causes of death 2000-2016, Department of Information, Evidence, and Research WHO, Geneva, 2018.

[5] Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences?. J Gastroenterol Hepatol. 2007;22:788–793. [CrossRef]

[6] Uroko R, Nwuke C, Agbafor A, Okwor J. Evaluation of in vitro antioxidants activities, hepatoprotective and haematological effects of ethanol extract of *Anthocleista vogelii* Stem Bark (AVSB) on carbon tetrachloride (CCL4) induced rats. Marmara Pharm J. 2021; 25(3): 258–270. [CrossRef]

[7] Indahsari NK, Masfufatun M, Devi EDR. Potensi ekstrak daun kelor (*Moringa oleifera*) sebagai hepatoprotektor pada tikus putih (*Rattus novergicus*) yang diinduksi parasetamol dosis toksik. Jurnal Ilmiah Kedokteran Wijaya Kusuma. 2018; 5: 58-66. [CrossRef]

[8] Toklu H, Şehirli Ö, Şahin H, Çetinel Ş, Yeğen BC, Şener G. Resveratrol supplementation protects against chronic nicotine-induced oxidative damage and organ dysfunction in the rat urogenital system. Marmara Pharm J. 2010; 14(1): 29–40. [CrossRef]

[9] Adewusi EA, Afolayan AJ. A review of natural products with hepatoprotective activity. J Med Plants Res. 2010; 4: 1318–1334.

[10] Susilawati E, Lister INE, Fachrial E. The protective effect of *Phyllanthus emblica* Linn. extract against doxorubicin-induced hepatotoxicity in rats. Pharmaciana. 2021; 11(1): 153–162. [CrossRef]

[11] Yayintas OT, İrkin LC. Bryophytes as hidden treasure. Journal of Scientific Perspectives. 2018; 2(1): 71–83. [CrossRef]

[12] Fadhilla R, Iskandar EA, Kusumaningrum D. Antibacterial activity of liverwort (*Marchantia paleacea*) extract on pathogenic and food spoilage bacteria "(article in Indonesian with an abstract in English)". Jurnal Teknologi dan Industri Pangan. 2012; 23(2): 126–131. [CrossRef]

[13] Zhang K, Cao H, Gao Y, Zhong M, Wang Y, Guan Y, Wei R, Jin L. *Marchantia polymorpha* L. flavonoids protect liver from CCl4-induced injury by antioxidant and gene-regulatory effects. Altern Ther Health Med. 2022; 28: 34–41.

[14] Jian-bo X, Feng-lian R, Xu M. Anti-hepatitis B virus activity of flavonoids from *Marchantia convoluta*. Iranian Journal of Pharmacology and Therapeutics. 2005; 4(2): 128–131.

[15] Hasballah K, Sarong M, Rusly R, Tantri K, Mulia VD. Acute toxicity and hepatotoxicity evaluation of methanol extract of root bark of *Calotropis gigantea* in Rats. Dhaka Univ J Pharm Sci. 2018; 17(2): 243-250. [CrossRef]

[16] Handayani S, Wirasutisna KR, Insanu M. Penapisan fitokimia dan karakterisasi simplisia daun jambu mawar (*Syzygium jambos* Alston). Jurnal Farmasi UIN Alauddin Makassar. 2017; 5(3): 174–179.

[17] Kurniawan JC, Suryanto E, Yudistira A. Analisis fitokimia dan uji aktivitas antioksidan dari getah kulit buah pisang goroho (*Musa acuminate* (L.)). Pharmacon. 2013; 2(3): 34–39.

[18] Wang L, Wang LN, Zhao Y, Lou HX, Cheng AX. Secondary metabolites from *Marchantia paleacea* calluses and their allelopathic effects on *Arabidopsis* seed growth. Nat Prod Res. 2013; 27(3): 274–276. [CrossRef]

[19] Asakawa Y. Liverworts-potential source of medicinal compounds. Curr Pharm Des. 2008; 14: 3067–88. [CrossRef]

[20] Xiao J, Jiang X, Chen X. Antibacterial, anti-inflammatory, and diuretic effect of flavonoids from *Marchantia convoluta*. Afr J Tradit Complement Altern Med. 2005; 2(3): 244–252.

[21] Novianto A. Hepatoprotector activity ethyl acetate fraction of Kenikir (*Cosmos caudatus*) on rat induced by paracetamol "(article in Indonesian with an abstract in English)". Indonesian Journal on Medical Science. 2016; 3(1): 35–41.

[22] Description of Curcuma FCT drug product at the Food and Drug Administration in Indonesia (BPOM RI). https://cekbpom.pom.go.id//home/produk/kdfq3nls24aa7v9vfqkld51ft7/all/row/10/page/1/order/4/DESC/search/1/Curcuma FCT (accessed June 9, 2022).

[23] Asri M, Kalsum K. Hepatoprotector test of fruit (*Solanum muricatum*) ethanol extract against male white rats induced by CCl_4 "(article in Indonesian with an abstract in English)". Media Kesehatan Politeknik Kesehatan Makassar. 2021; 16(1): 167–172.

[24] Hariyanti LPD, Wardhana AANW, Indriyani NKS, Putri IAPY. Pengaruh pemberian ekstrak etanol daun binahong (*Anredera scandens* (L.) Moq.) terhadap histopatologi hati mencit jantan galur Balb/c yang diinduksi dengan karbon tetraklorida. Jurnal Farmasi Udayana. 2017; 6(1): 39–42. [CrossRef]

[25] Suriani S, Firawati F, Pertiwi S. Uji aktivitas hepatoprotektor ekstrak kubis ungu (*Brassica oleracea* var. Capitata L.) terhadap hewan uji kelinci (*Oryctolagus cuniculus*) yang diinduksi karbon tetraklorida (CCl_4). Jurnal Farmasi UIN Alauddin Makassar. 2013; 7(1): 17-25.

[26] Liem S, Levita J. Review of hepatoprotector of rosela (*Hibiscus sabdariffa*): activity, mechanism of action, and toxicity. Jurnal Farmasi Galenika (Galenika Journal of Pharmacy). 2017; 3(2): 103–117. [CrossRef]

[27] Freitag AF, Cardia GFE, Da Rocha BA, Aguiar RP, Silva-Comar FMDS, Spironello RA, Grespan R, Caparros-Assef SM, Bersani-Amado CA, Cuman RKN. Hepatoprotective effect of silymarin (*Silybum marianum*) on hepatotoxicity induced by acetaminophen in spontaneously hypertensive rats. Evid Based Complement Alternat Med. 2015; 2015: 1-8. [CrossRef]

[28] Olaleye MT, Akinmoladun AC, Ogunboye AA, Akindahunsi AA. Antioxidant activity and hepatoprotective property of leaf extracts of *Boerhaavia diffusa* Linn against acetaminophen-induced liver damage in rats. Food Chem Toxicol. 2010; 48(8-9): 2200–5. [CrossRef]

[29] Seif HSA. Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. Beni Suef Univ J Basic Appl Sci. 2016; 2016: 1-13. [CrossRef]

[30] Chaphalkar R, Apte KG, Talekar Y, Ojha SK, Nandave M. Antioxidants of *Phyllanthus emblica* L. bark extract provide hepatoprotection against ethanol-induced hepatic damage: A comparison with silymarin. Oxid Med Cell

Longev. 2017; 2017: 1-10. [CrossRef]

[31] Sabri E, Ilyas S, Prasetiawan E. The effect of n-hexane extract of andaliman (*Zanthoxylum acanthopodium* DC.) fruit in the liver tissues of mice during post implantation of pregnancy. J Phys Conf Ser. 2018; 1116(5): 1-5. [CrossRef]

[32] Ilyas S, Tanjung M, Nurwahyuni I, Pasaribu FY. Effect of mahkota dewa ethanolic extracts (*Phaleria macrocarpa* Scheff.) to hepar histology of female rat (*Rattus novergicus*) in preeclampsia. IOP Conf Ser Earth Environ Sci. 2019; 305(1): 1-8. [CrossRef]

[33] Luedde T, Kaplowitz N, Schwabe RF. Cell death and cell death responses in liver disease: Mechanisms and clinical relevance. Gastroenterology. 2014; 147(4): 765-83. [CrossRef]

[34] Gökbulut A, Satilmiş B, Batçioğlu K, Çetin B, Şarer E. Antioxidant activity and luteolin content of *Marchantia polymorpha* L. Turk J Biol. 2012; 36: 381–5. [CrossRef]

[35] Sinam G, Sahu V, Pandey N, Asthana AK. Effect of arsenic on biochemical and antioxidant enzymes in two species of *Marchantia* L. (Marchantiophyta): Role of enzymes in stress acclimatization. Journal of Environmental and Agricultural Sciences. 2016; 8: 79–85.

[36] Qian D, Florenly F, Liena L, Purba DR. Effectiveness of ethanol extract mangosteen peel (*Garcinia mangostana* L.) in accelerating post-tooth extraction healing in Wistar rats. Budapest Innternational Research in Exact Sciences (BirEx) Journal. 2021; 4(1) :22–30. [CrossRef]

[37] Şehirli Ö, Şener G. Protective effect of erdosteine against naphthalene-induced oxidative stress in rats. Marmara Pharm J. 2010; 14(2): 67–73. [CrossRef]

[38] Oktavia S, Ifora I, Suhatri S, Susanti M. Uji aktivitas hepatoprotektor ekstrak daun sirih hijau (*Piper betle* Linn.) terhadap kerusakan hati yang diinduksi parasetamol. Jurnal Farmasi Higea. 2017; 9(2): 109–117.

[39] Adyttia A, Untari EK, Wahdaningsih S. Efek ekstrak etanol daun *Premna cordifolia* terhadap malondialdehida tikus yang dipapar asap rokok. Pharmaceutical Sciences and Research. 2014; 1(2): 104–115. [CrossRef]

[40] Sebayang R, Idawati Y, Sinaga H. Analisis lactat dehydrogenase dalam serum darah menggunakan sentrifugasi. Jurnal Keperawatan Silampari. 2020; 4(1): 274–280.

[41] Rahman S, Kosman R, Siamea AC. Efek hepatoprotektor dari ekstrak etanol daun johar (*Cassia siamea* lamk.) pada tikus (*Rattus norvegicus*). Jurnal Ilmiah As-Syifaa. 2017; 9(2): 131-136.

[42] Wahidah LK, Triyandi R, Indriani R. Fraksi air daun ubi jalar (*Ipomea batatas* (L.) Lam.) sebagai hepatoprotektor terhadap tikus putih jantan yang diinduksi paracetamol. Jurnal Farmasi Lampung. 2019; 8(1): 39–46. [CrossRef]