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UHPLC–QTOF–MS Metabolic Profiling of *Marchantia polymorpha* and Evaluation of Its Hepatoprotective Activity Using Paracetamol-Induced Liver Injury in Mice

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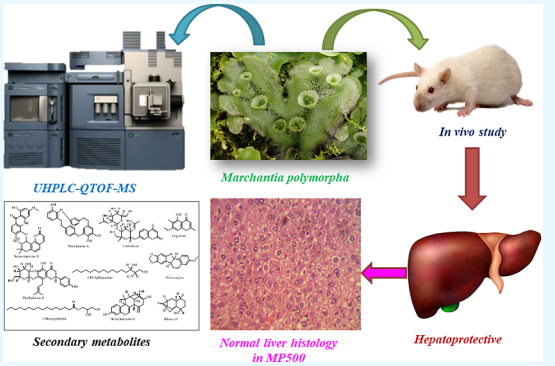
ABSTRACT: *Marchantia* species were traditionally used to treat liver failure. *Marchantia polymorpha* chloroform extract showed a marked hepatoprotective activity in a dose-dependent manner in paracetamol-induced extensive liver damage in mice. At a dose of 500 mg/kg (MP-500), it resulted in a reduction in aspartate transaminase by 49.44%, alanine transaminase by 44.11%, and alkaline phosphatase by 24.4% with significant elevation in total proteins by 58.69% with respect to the diseased group. It showed significant reductions in total bilirubin, total cholesterol, triglycerides, low density lipoprotein (LDL), very LDL, total lipids, and to high density lipoprotein ratio (CH/HDL) by 53.42, 30.14, 35.02, 45.79, 34.74, 41.45, and 49.52%, respectively, together with a 37.69% increase in HDL with respect to the diseased group. It also showed an elevation of superoxide dismutase by 28.09% and in glutathione peroxidase by 81.83% in addition to the reduction of lipid peroxidation by 17.95% as compared to the paracetamol only treated group. This was further supported by histopathological examination that showed normal liver architecture and a normal sinusoidal gap. Metabolic profiling by ultrahigh performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometer (UHPLC−QTOF/MS) led to the tentative identification of 28 compounds belonging to phenols, quinolones, phenylpropanoid, acylaminosugars, terpenoids, lipids, and fatty acids to which the activity was attributed. Four compounds were detected in the negative ionization mode which are neoacrimarine J, marchantin A, chitobiose, and phellodensin F, while the rest were detected in the positive mode. Thus, it can be concluded that this plant could serve as a valuable choice for the treatment of hepatotoxicity that further consolidated its traditional use.



INTRODUCTION

Liver is the body's largest organ that performs a number of crucial tasks, including metabolism of protein, carbohydrates, and fat; the process of detoxification; the production and secretion of several enzymes; as well as the production of bilirubin.[1,2] Numerous infections, medications, long-term diabetes, as well as alcohol in addition to many poisons can act upon this organ, resulting in its deterioration with the concomitant appearance of liver necrosis and cirrhosis.[3]

A large number of naturally occurring herbal products showed a prominent effect on the liver in spite of the fact that many of their bio-active constituents are still unknown. They were frequently employed for the treatment of liver disorders due to their effectiveness, fewer adverse effects and inexpensive cost compared to synthetic agents. They exerted their effect on liver via prohibition and scavenging of free radicals by their antioxidant potential that facilitates the prevention of infections and degenerative disorders as well.[4]

Bryophytes are often a group of lower green land plants without well-developed vascular systems with a dominating leafy generation called gametophyte (haploid generation); meanwhile, their sporophytic (diploid generation) is a spore-bearing and many times get along with the gametophyte for their whole life cycle. The bryophytic plants generally exist in less significant and low-pitched noticeable places and are frequently disregarded by human beings.[5−7] Hence, bryophytes are less explored compared to vascular plants and remained under-examined in many areas particularly their medicinal importance.

Marchantia polymorpha L. is a thallus liverwort of class Hepaticae with green to brown or purple colored, hexangular marks on ramified branches of about 10 cm long and up to 2

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cm in width. It maturates on moist soil, damp rocks, stream's banks, puddles, and peat bogs. Undersides, they are covered by many root-like rhizoids and give rise to reproductive structures known as gametophores. Female gametophore plants contain a stalk with rays containing archegonia which give rise to ova. Male gametophores have a flat disc bearing antheridia that develop sperms. It has been observed that people of Himalayan areas make use of a mixture of ashes made from *M. polymorpha* and *Marchantia palmata* plants, blended with honey and a small quantity of fat for healing cuts, burns, and other skin injuries.[7−9] Besides, in numerous classical Greek references and medical documents, the *Marchantia* species were used to cure open wounds, prevent bacterial infections, treat external wounds that are inflammatory or painful, work as snake antivenom, and treat liver failure.[10]

Phytoconstituents existing in *M. polymorpha* are volatile metabolites belonging to terpenoids such as thujopsene and β-chamigrene in addition to aromatic compounds, comprising bibenzyls and bisbibenzyls.[11,12] Regarding its biological activity, *M. polymorpha* exhibited a potent biological potential particularly antifungal, antibacterial, anti-inflammatory, antiviral, and anti-cancer as well that is mainly relied upon its metabolites represented by marchantin A, marchantin B, neomarchantin A, riccardin H, and perrottetin E.[13] Moreover, a recent study performed on a *M. polymorpha* L. extract in vitro revealed significant antioxidant and tyrosinase inhibition potential.[14] Besides, endophytes isolated from *M. polymorpha* showed antiviral and anticancer potential owing to their volatile cyclic dipeptides.[15]

Tracing the current literature, nothing was found regarding the hepatoprotective potential of *M. polymorpha* L. as well as its mechanisms of action. Meanwhile, the search for an alternative therapy for treating of hepatic disorders, with minimum side consequences particularly derived from natural sources is considered mandatory worldwide. Thus, the current study aimed to comprehensively validate the hepatoprotective potential of the chloroform extract of *M. polymorpha* L. whole body using paracetamol (PCM)-induced liver injury in mice with subsequent measurement of the levels of oxidative stress markers and liver biomarkers that was further supported by histopathological studies. Besides, metabolic profiling of *M. polymorpha* bioactive chloroform extract was performed using ultra high performance liquid chromatography coupled with a mass Q-TOF spectrometer (UPLC/MS) to further correlate between the bioactivity and the prevailing secondary metabolites to consolidate folk employment of bryophytic plant species such as *M. polymorpha* L as a hepatoprotective agent.

MATERIALS AND METHODS

Plant Material. Whole *M. polymorpha* L. plants were obtained from Khanspure and Nathigalli areas (in the northern region of Pakistan). The specimen of the plant species was identified and authenticated by Dr. Zaheer-ur-Khan, a plant taxonomist from Botany Department, Government College University Lahore. The plant specimen was allocated in the herbarium of Department of Pharmacy, University of Central Punjab, Lahore, Pakistan with Voucher number of Cog-010 for further reference. The plant material was carefully dried, garbled, pulverized, and placed in a glass container.

Crude Plant Extracts Preparation. The ground plant material (4 Kg) was macerated successively with three major solvents, namely, *n*-hexane, chloroform, and methanol. 8 L of each of the solvent were used consecutively and the plant material was kept in each solvent for 7 days. Each solvent extract was percolated thoroughly with muslin cloth, followed by filtration through Whatman−1 filter paper and the filtration operation was repeated twice or three times to get a maximum yield of each. All the solvent extracts were subjected to concentration using rotary evaporator under reduced pressure.[16] Hexane extract was dark-orange, whereas the chloroform extract was dark-brown, whereas the methanol extract was greenish-black in color. Each of the extracted material was further dried in an oven at 37 °C to get the semisolid extract. Each extract was labeled and preserved in an airtight container at 25−30 °C.

Chemicals and Drugs. Silymarin was purchased from local pharmacy and PCM powder was obtained from Pacific Pharma Limited, Lahore, Pakistan. The kits for the assay of serum enzymes were provided by Sigma-Aldrich. Different doses of chloroform extract (250 and 500 mg/kg) were prepared in 10% Tween20 solution, while PCM 250 mg/kg were prepared in distilled water (DW) for administration. Silymarin (50 mg/kg) was also prepared in DW.

UPLC/MS Metabolic Profiling of the Chloroform Extract of *M. polymorpha* L. Metabolic profiling of the chloroform extract of *M. polymorpha* L. was done using UPLC coupled with mass Q-TOF spectrometer (UPLC/MS). Agilent 6520 Accurate-Mass Q-TOF mass spectrometer (MS) with twin ESI 18 sources and Agilent 1290 Infinity 17 LC system UHPLC was employed. Agilent Zorbax Eclipse XDB-C18, narrow19 bore 2.1 × 150 mm, 3.5 μm (P/N: 930990-902) were the column's technical specifications. Temperatures for the column and auto-sampler 20 were kept at 25 and 4 °C, respectively. The flow rate was 0.5 mL/min, whereas formic acid, concentrations of 0.1% in water and 0.1% in acetonitrile were the mobile phases employed; meanwhile, the injection's volume was 1 micro-L. There was a 25 min run and a 5 min recovery period. Electrospray ion source in both the negative mode and positive mode was employed, and full scan MS analysis was performed over the m/z 100−1000 range. Nitrogen was provided at flow rates of 25 and 600 L/h, respectively, for nebulizing and drying gas purposes. 350 °C was the temperature of the drying gas, whereas the voltage for fragmentation was calibrated to 125 V. An analysis was conducted using a 3500 V capillary voltage. Agilent Mass Hunter Qualitative Analysis B.05.00 was used to process the data (Method: Metabolomics3 2017-00004.m). Compounds were identified using the following search parameters in the database: 4 METLIN AM PCDL-N- 170502.cdb: 5 ppm match tolerance ions with positive charges include the following: +H, +Na, +NH4, and −H.

In Vivo Hepatoprotective Evaluation of *M. polymorpha* L. Chloroform Extract. *Experimental Animals.* Studies were conducted on Swiss albino male mice weighing 25−30 g. The animals were laid in Plexiglas cages (47 × 34 × 18 cm^3) in the Research Laboratory of Pharmacology and Physiology, Faculty of Pharmacy, University of the Punjab Lahore, Pakistan. The laboratory temperature was sustained at 26 ± 2 °C and humidity was maintained at 50−55% along with 12 h light−dark cycle. All the animals were adapted for 7 days with the experimental status and fed with standard animal food and water. The standard diet was prepared according to Institutional guidelines and was composed of 20% fat (5% sunflower oil + 14.5% cottonseed oil + 0.5% linoleic acid), 16% proteins in addition to calcium (5%), amino acids mixture

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Table 1. UHPLC−QTOF−MS Metabolic Profiling of the Chloroform Extract of *Marchantia polymorpha* L. in the Negative Ionization Mode

peak no	RT (min)	base peak (m/z)	compound name	compound class	molecular formula	mass	refs
1	14.03	516.1303	Neoacrimarine J	Quinolines	$C_{28}H_{23}NO_9$	517.1376	44
2	14.224	439.1560	MarchantinA	Phenylpropanoid	$C_{28}H_{24}O_5$	440.1632	38
3	14.246	423.1560	Chitobiose	Acylaminosugars	$C_{16}H_{28}N_2O_{11}$	424.1679	45
4	14.897	537.1527	Phellodensin F	Glycoside	$C_{26}H_{30}O_{10}$	502.1836	39

Table 2. UHPLC−QTOF−MS Metabolic Profiling of the Chloroform Extract of *Marchantia polymorpha* L. in the Positive Ionization Mode

peak no	RT (min)	base peak (m/z)	compound name	compound class	molecular formula	mass	refs
1	9.255	197.1166	4-(2-hydroxypropoxy)-3,5-dimethyl-Phenol	phenol	$C_{11}H_{16}O_3$	196.1093	46
2	11.482	244.1901	dihydrojasmonic acid, methyl ester		$C_{13}H_{22}O_3$	226.1562	47
3	12.153	274.2733	C16 sphinganine	lipid	$C_{16}H_{35}NO_2$	273.2659	48
4	12.454	228.1948	10-tridecynoic acid	fatty acid	$C_{13}H_{22}O_2$	210.1608	49
5	12.518	158.1533	2,6-nonadien-1-ol	alcohol	$C_9H_{16}O$	140.1193	50
6	12.58	221.0802	eugenitin	phenolic compound	$C_{12}H_{12}O_4$	220.0731	51
7	15.159	277.2149	8E-tetradecenyl acetate	carboxylic ester	$C_{16}H_{30}O_2$	254.2257	52
8	15.471	299.0900	pterocarpin		$C_{17}H_{14}O_5$	298.083	53
9	15.664	219.1735	(+)-arnicenone	sesquiterpene	$C_{15}H_{22}O$	218.1662	54
10	15.842	279.2301	2-hexyl-decanoic acid	fatty acid	$C_{16}H_{32}O_2$	256.2407	55
11	15.888	398.2527	[6]-gingerdiol 3,5-diacetate	carboxylic ester	$C_{21}H_{32}O_6$	380.2191	56
12	16.056	343.1534	deoxymiroestrol	phytoestrogen	$C_{20}H_{22}O_5$	342.1464	57
13	16.232	221.1902	ishwarol	sesqiterpene alcohol	$C_{15}H_{24}O$	220.1828	58
14	16.439	343.1532	deoxymiroestrol		$C_{20}H_{22}O_5$	342.1460	
15	16.565	425.3770	moretenone	terpenoids	$C_{30}H_{48}O$	424.3693	59
16	16.566	407.3660	4,4′-diapophytofluene	carotenoid triterpenoid	$C_{30}H_{46}$	406.3586	60
17	16.74	456.2164	hericenoneB	benzaldehydes	$C_{27}H_{31}NO_4$	433.2271	61
18	16.907	279.1589	emmotinA		$C_{16}H_{22}O_4$	278.1516	40
19	17.508	459.3821	longispinogenin	triterpenoid	$C_{30}H_{50}O_3$	458.3748	62
20	18.305	320.2578	*N*-hydroxy arachidonoyl amine	fatty acid	$C_{20}H_{33}NO_2$	319.2505	63
21	18.519	372.3098	1-linoleoyl glycerol	fatty acid	$C_{21}H_{38}O_4$	354.2756	64
22	19.16	322.2732	(−)-isoamijiol	diterpene	$C_{20}H_{32}O_2$	304.2383	65
23	19.48	331.2827	1-monopalmitin	fatty acid	$C_{19}H_{38}O_4$	330.2755	66
24	20.317	384.3455	*N*-stearoyl valine		$C_{23}H_{45}NO_3$	383.3380	67

(40%), vitamins mixture (1%), minerals (7%), as well as casein (11%). All the experimental protocols were approved by the Pharmacy Animal Ethics Committee with approval no. 2101 in the Faculty of Pharmacy, University of the Punjab Lahore, Pakistan.

Experimental Protocol. Mice were divided into five groups, each group consisting of animals. Group I served as control and received DW only. Group II was the control diseased group that orally administered with 250 mg/kg of PCM. Meanwhile, group III served as the standard group that was orally administered with 50 mg/kg silymarin in addition to 250 mg/kg of PCM. However, groups IV and V were the examined extracts groups in which the animals were orally administered with 250 and 500 mg/kg of *M. polymorpha* chloroform extract (MP-250) and (MP-500), respectively, in addition to oral administration of 250 mg/kg of PCM for 14 days.[17] Mice were anesthetized with ketamine + xylazine and blood was drawn for biochemical evaluation by heart puncture following 15 days of therapy. Blood was centrifuged at 4000 rpm for 15 min at room temperature to obtain the serum.

Biochemical and Oxidative Stress Markers Evaluation. All liver function tests including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total proteins (TP) as well as lipid profile parameters comprising total bilirubin (TB), total cholesterol (TC), triglycerides (TGs), low density lipoprotein (LDL), very LDL (VLDL), total lipids (TL), and cholesterol to high density lipoprotein ratio (CH/HDL) and HDL were evaluated using serum analysis as previously reported by Parvez et al.[18] However, for antioxidant investigations, 10% liver homogenate was employed and oxidative stress parameters were assessed including superoxide dismutase (SOD), lipid peroxidation (LPO), and glutathione peroxidase (GPx) was analyzed using the previously reported methods employed by Rajkapoor, et al.[19]

Histopathological Evaluation. Liver specimens were dehydrated, cleaned, and embedded in paraffin blocks after being stored in 10% formalin solution. To report histology, paraffin slices were cut and stained with hematoxylin and eosin dye.[20]

Statistical Analysis. Results were represented as mean ± SD (n = 6). Two-way ANOVA followed by post-hoc Dunnett test was performed using Graph Pad Prism (San Diego, CA, USA) software. p-value of less than 0.05 was considered statistically significant.

RESULTS

UHPLC−QTOF−MS Metabolic Profiling of the Chloroform Extract of *M. polymorpha* L. Metabolic profiling of the chloroform extract of *M. polymorpha* L. using UPLC

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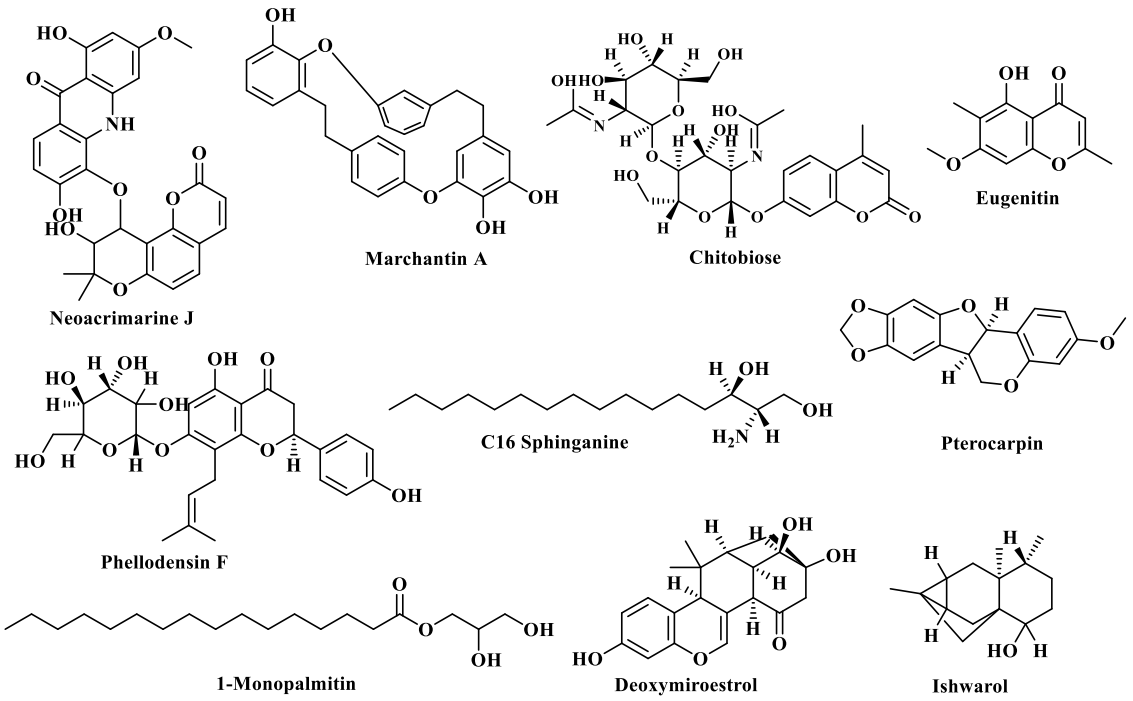

Figure 1. Scheme showing the chemical structure of major identified compounds from the *Marchantia polymorpha* chloroform extract using UHPLC−QTOF−MS.

coupled with quadrupole time of flight MS (UHPLC−QTOF) in both positive and negative ionization modes. This led to the tentative identification of 28 compounds; four of which were identified in the negative ionization mode whereas the rest were determined from the positive ionization mode, as illustrated in Tables 1 and 2. These compounds belong to various classes including benzoic acids and their derivatives, phenols, oligothiophenes, quinolones, phenylpropanoid, acylaminosugars, terpenoids, lipids, and fatty acids. Tentative assignment of the detected metabolites was performed based upon comparing the mass of the existing metabolites in both positive and negative and ionization modes with previously reported data (references were illustrated in Tables 1 and 2) together with public online databases such as pubchem and Massbank. A scheme showing the chemical structures of the identified compounds in the chloroform extract of *M. polymorpha* L. is represented in Figure 1.

In Vivo Hepatoprotective Evaluation of *M. polymorpha* L. Chloroform Extract. *Effect on Liver Stress Markers.* Paracetamol-induced an extensive liver damage in mice evidenced by a pronounced elevation in ALT, AST, and ALP levels estimated by 144.6, 82.57, and 55.22%, respectively, with concomitant reduction in TP by 24.68% as compared to the control group. In contrast, administration of (MP-250) and (MP-500) resulted in a significant amelioration of the extensive liver damage as revealed through the reduction of ALT by 44.05 and 49.49%, respectively, AST by 35.98 and 44.07%, respectively, ALP by 18.93 and 23.08%, respectively, in addition to a significant elevation in TP by 45.21 and 54.47%, respectively, with respect to the diseased group that received paracetamol only. They approach in this respect silymarin that revealed 51.49, 52.85, and 24.90% reduction in ALT, AST, and ALP levels, respectively, in addition to 44.83% elevation in TP (Figure 2).

Effect on Lipid Profile. Besides, oral administration of paracetamol induced an extensive alteration in lipid profile manifested by an abrupt elevation in TB, TC, TG, LDL, VLDL, TL, and CH/HDL estimated by 80.49, 84.44, 138.43, 180.7, 181.64, 136.87, and 281.21%, respectively, together with a significant reduction in HDL by 51.47% as compared to the control group. However, administration of (MP-250) caused a notable reduction in TB, TC, TG, LDL, VLDL, TL, and CH/HDL by 30.81, 28.88, 29.72, 43.62, 27.51, 4.05, and 40.87%, respectively, in addition to 20.93% elevation in HDL as compared to the diseased group. Meanwhile, oral administration of (MP-500) resulted in a significant reduction in TB, TC, TG, LDL, VLDL, TL, and CH/HDL by 51.35, 28.96, 35.07, 45.77, 34.79, 41.33, and 48.26%, respectively, together with 37.49% increase in HDL with respect to the diseased group. These results approached silymarin, the standard hepatoprotective agent that showed 54.26% elevation in the HDL level with 62.16, 34.73, 40.11, 51.55, 43.50, 29.90, and 57.68% decline in TB, TC, TG, LDL, VLDL, TL, and CH/HDL, respectively, in comparison to the diseased group (Figures 3 and 4).

Effect on Oxidative Stress Markers. In addition, oral administration of paracetamol triggers a pronounced elevation in oxidative stress elaborated by the diseased group expressed by a decline in endogenous antioxidants estimated by 31.17 and 58.07%%, for SOD and GPx, respectively, with concomitant elevation in LPO by 40.74%. In contrast, administration of silymarin, (MP-250) and (MP-500) resulted in an elevation of SOD by 33.56, 22.01, and 28.95%, respectively, with concomitant increase in GPx by 114.14, 76.11, and 82.42%, respectively, compared to the diseased group. Besides, they reduced LPO by 22.39, 11.59, and 14.26%, respectively, as compared to paracetamol only treated groups (Table 3).

Histopathological Examination. M. polymorpha extracts elicited a pronounced amelioration in the liver histology, as presented in Figure 5. According to the histopathological studies, the control group showed normal hepatocyte (white

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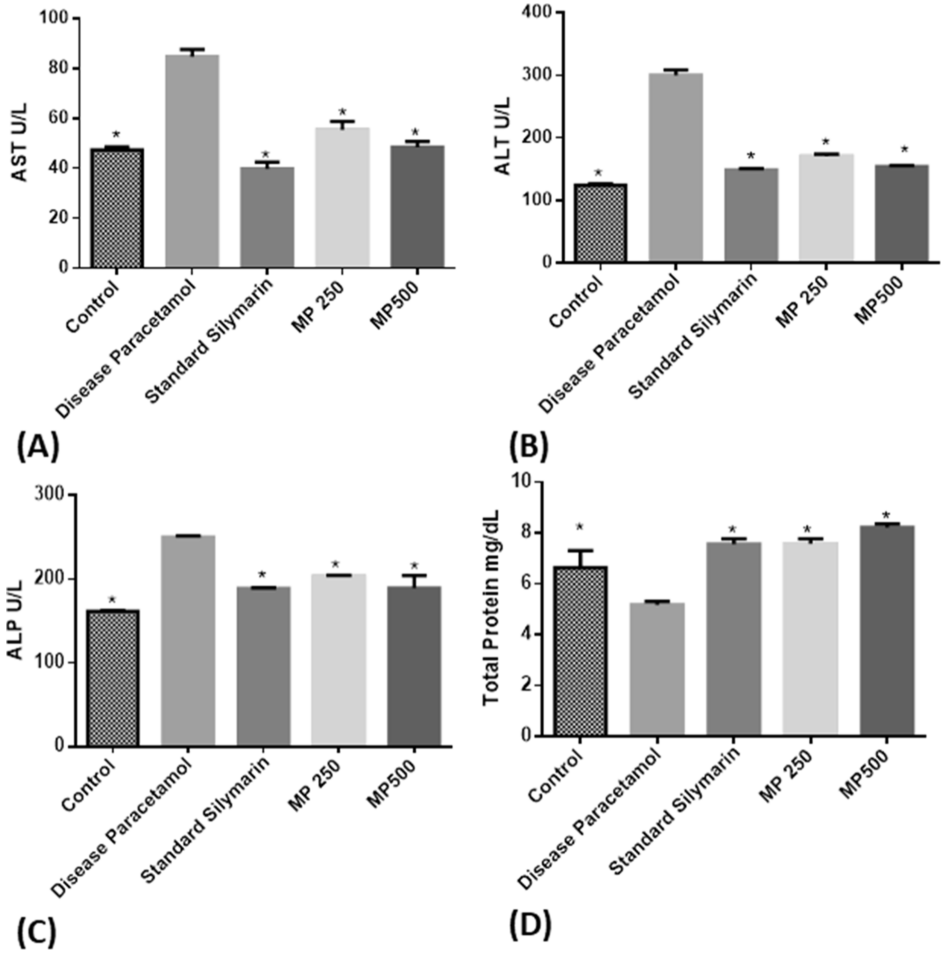

Figure 2. Effect of *Marchantia polymorpha* at doses of 250 and 500 mg/kg and silymarin (50 mg/kg) on liver biomarkers; (A) AST; (B) ALT; and (C) AST, and (D) total protein in PCM-induced hepatotoxicity in mice; results are expressed in mean ± SD; $n = 5$. *$P < 0.05$ considered to be significant from PCM. One-way ANOVA variance followed by Dunnett's multiple comparison tests was performed using graph pad prism software.

arrow) and normal sinusoids (blue arrow), whereas paracetamol intoxicated mice revealed significant vascular degeneration and centrilobular necrosis in hepatocytes in addition to hepatocyte ballooning and degeneration (white arrow) together with abnormal sinusoid architecture (blue arrow). When compared to control, administration of various doses of *M. polymorpha* extract led to mild degenerative alterations in hepatocytes and sinusoids. The MP-250-treated group showed normal hepatocyte architecture (white arrow) and a mild shrinkage of sinusoid (blue arrow), whereas MP 500 revealed a better amelioration in the liver histology manifested by normal hepatocyte architecture (white arrow) and a normal sinusoidal gap (blue arrow). Meanwhile, oral administration of silymarin, standard hepatoprotective agent, showed preserved hepatocyte architecture (white arrow) and a mild change in sinusoids (blue arrow) (Figure 5).

DISCUSSION

Ethnopharmacological investigations showed that many plants possess hepatoprotective properties and were employed traditionally in various regions of the world to treat various liver ailments.[21,22] The current research assessed the hepatoprotective potential of *M. polymorpha*, which further consolidates its traditional usage. The antioxidant components existing in plants as phenols, phenolic diterpenes, are mainly responsible for their pharmacological properties.[23,24] Metabolic profiling performed using UHPLC−MS revealed the richness of the plants with secondary metabolites with antioxidant potential to which its hepatoprotective effect was attributed. Paracetamol (Acetaminophen), a popular analgesic and febrifuge medication was highly reputed to cause severe liver injury in both experimental animals and in humans.[25]

Paracetamol-induced hepatotoxicity was used as a reliable approach for screening hepatoprotective drugs. The liver is the primary site for paracetamol metabolism, and the kidneys are responsible for excreting it after its conjugation with glucuronide and sulfate.[26] It is known that a portion of acetaminophen is metabolized via cytochrome P450 pathway to *N*-acetyl-*p*-benzoquinamine, a highly poisonous metabolite that is typically conjugated with glutathione and eliminated in the urine. Acetaminophen intoxication depletes glutathione reserves, resulting in the buildup of NAPQI, mitochondrial malfunction, and the emergence of acute hepatic necrosis.[26]

The toxic metabolites (*N*-acetyl-*p*-benzoquineimine) can alkylate and oxidize intracellular GSH, which causes liver GSH depletion. Increased LPO is then caused by the abstraction of hydrogen from a polyunsaturated fatty acid, which ultimately causes liver damage from higher paracetamol doses.[27] Reactive metabolites can cause early cell stress in a variety of ways, such as through diminishing glutathione (GSH) levels or by attaching to enzymes, lipids, nucleic acids, and other cell components. Studies showed that compounds that affect P450

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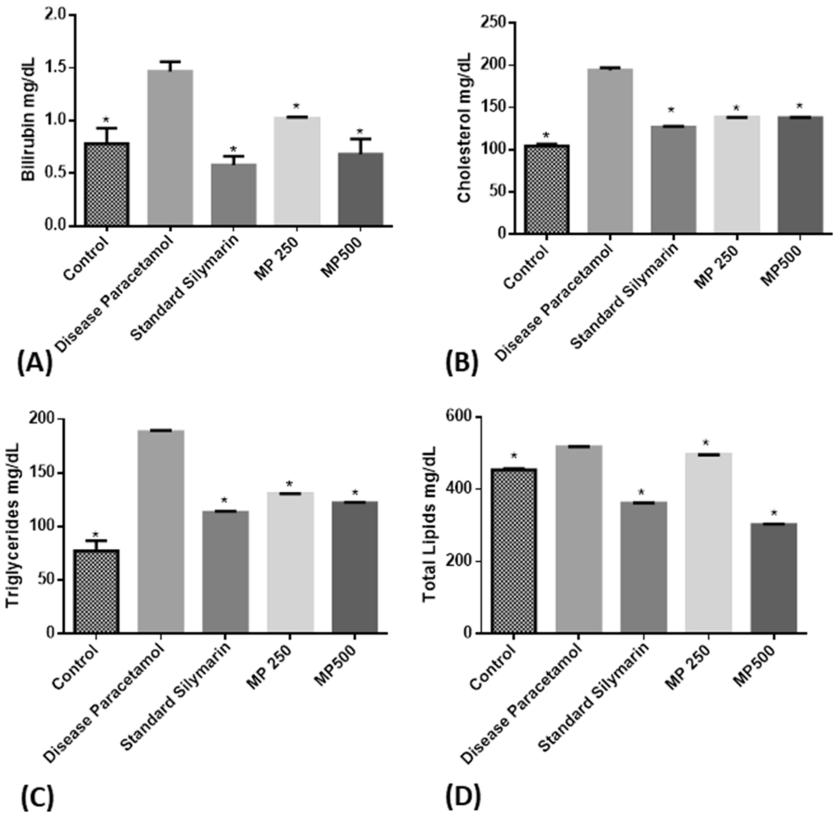

Figure 3. Effect of *Marchantia polymorpha* at doses of 250 and 500 mg/kg and silymarin (50 mg/kg) on the lipid profile; (A) bilirubin; (B) cholesterol; (C) triglycerides; and (D) total lipids in PCM-induced hepatotoxicity in mice; results are expressed in mean ± SD; $n = 5$ *$P < 0.05$ considered to be significant from PCM. One-way ANOVA variance followed by Dunnett's multiple comparison tests was performed using graph pad prism software.

activity can prevent the liver damage caused by PCM.[28] The monitoring of enzyme levels like AST and ALT is frequently utilized in the assessment of liver damage caused by acetaminophen. The enzyme is released into circulation by necrosis or membrane injury, and as a result, it can be detected in the serum. Hepatocytes' mitochondria are the primary location of AST. ALT is a better measure for identifying liver damage since it is more specific to the liver in addition, damage of liver cells is also linked to serum ALP and bilirubin levels.[29] When acetaminophen was administered, it significantly increased the levels of several enzymes, including AST, ALT, ALP, GGTP, and TB, and decreased TP relative to the control group. The increased levels of the blood marker enzymes AST, ALT, ALP, and bilirubin were counteracted by co-administering the chloroform extracts of the plant under investigation in a dose-dependent manner. The extract may have the ability to stabilize membranes, prevents the leaking of intracellular enzymes, which would otherwise cause higher serum enzyme levels in acetaminophen-induced liver injury. This is consistent with the widely held belief that the repair of the hepatic parenchyma and the regeneration of the hepatocytes cause serum levels of transaminases to revert to normal.[30−32] Toxic metabolite NAPQI frequently causes cell death, organ damage, and covalent alteration of cellular target proteins.[33] Effective regulation of ALP, bilirubin, and TP levels suggested that the hepatic cells' secretory system was improved.[33] Any hepatoprotective medication's effectiveness depends on its ability to either mitigate the negative effects or bring back the normal physiology of the liver after being interrupted by a hepatotoxin.

Both silymarin (50 mg/kg) and the plant extract (250 and 500 mg/kg) reduced acetaminophen-induced raised enzyme levels in the test groups, indicating the preservation of the structural integrity of the hepatocyte cell membrane or the regeneration of damaged liver cells. The rise in liver LPO caused by acetaminophen suggested an increased LPO causing tissue damage and failure of the antioxidant defense mechanism to stop the creation of too many free radicals. These alterations are significantly reversed by *M. polymorpha* treatment highlighting that the antioxidant impact of *M. polymorpha* is most likely the cause of its hepatoprotective effects.[34] Superoxide dimutase (SOD) enzyme activity reduction is a sensitive indicator of hepatocellular injury and is the most sensitive enzymatic signal in liver injury. According to reports, SOD is among the most crucial enzymes in the body's enzymatic antioxidant defense system. As a result, the radical's harmful effects are lessened since it scavenges the superoxide anion to produce hydrogen peroxide. The liver's reactive free radical-induced oxidative damage is decreased as a result of *M. polymorpha* considerable increase in hepatic SOD activity.[35]

One of the most prevalent tripeptides, nonenzymatic biological antioxidants in the liver is glutathione. It keeps membrane protein thiols intact and eliminates free radical species like hydrogen peroxide and superoxide radicals.[36] It also serves as a GPx substrate. In mice treated with acetaminophen, a lower amount of GSH is connected to increased LPO. The level of GPx and GST was significantly ($P < 0.05$) and dose-dependently elevated after administration of *M. polymorpha* extract.[36] Additionally, the hepatoprotective

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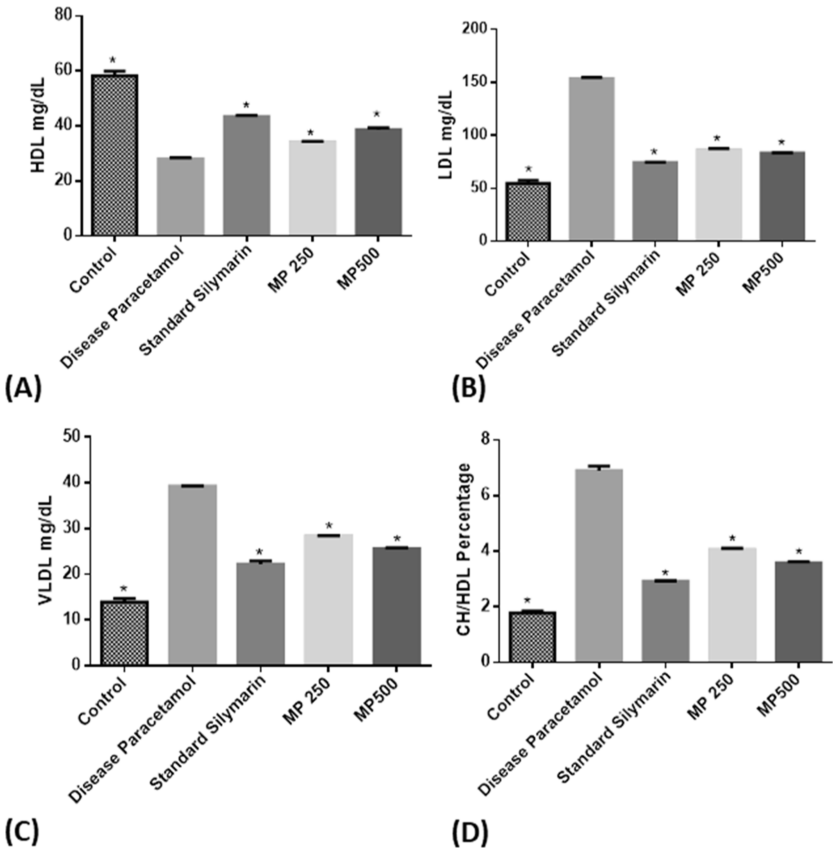

Figure 4. Effect of *Marchantia polymorpha* at doses of 250 and 500 mg/kg and silymarin (50 mg/kg) on lipid profile; (A) HDL; (B) LDL; (C) VLDL; and (D) CH/HDL percentage in PCM-induced hepatotoxicity in mice; results are expressed in mean ± SD; $n = 5$. *$P < 0.05$ considered to be significant from PCM. One-way ANOVA variance followed by Dunnett's multiple comparison tests was performed using graph pad prism software.

Table 3. Effect of *Marchantia polymorpha* at Doses of 250 and 500 mg/kg and Silymarin (50 mg/kg) on the Antioxidant Level in PCM-Induced Hepatotoxicity in Mice[a]

parameters	control	PCM	silymarin	MP-250	MP-500
SOD (ng/mL)	25.54 ± 0.14*	17.58 ± 0.19	23.48 ± 0.38*	21.45 ± 0.25*	22.67 ± 0.26*
GPx (ng/mL)	37.44 ± 0.19*	15.70 ± 0.27	33.62 ± 1.42*	27.65 ± 4.23*	28.64 ± 0.26*
LPO (ng/mL)	28.62 ± 0.08*	40.28 ± 0.10	31.26 ± 0.69*	35.61 ± 1.24*	34.52 ± 2.65*

[a]Results are expressed in mean ± SD; $n = 5$. *$P < 0.05$ considered to be significant from PCM. One-way ANOVA variance followed by Dunnett's multiple comparison tests was performed using graph pad prism software.

efficacy of *M. polymorpha* extract was supported by the histological findings that greatly contributed to the counteracting of the damaged liver architecture. Acetaminophen caused extensive vascular degenerative alterations and centrilobular necrosis in hepatocytes. Administration of different doses of the chloroform extract of *M. polymorpha* caused only minor degenerative alterations and no centrilobular necrosis, showing that the extract was effective at protecting the liver. The investigated plant extract has hepatoprotective and antioxidant properties that regulate cellular permeability, stability, and decrease oxidative stress. Numerous studies have shown that some flavonoids, triterpenoids, and steroids have antioxidant characteristics that protect the liver.[37] Major compounds like marchantin A,[38] phellodensin F,[39] 2,2,4,4,-tetramethyl-6-(1-oxopropyl)-1,3,5-cyclohexanetrione emmotin A[40] identified in *M. polymorpha* extract using UHPLC−MS could be greatly attributed to its major role in hepatoprotective activity. Marchantin A previously reported to possess a potent anti-inflammatory activity that undoubtedly ameliorate hepatic inflammation and necrosis.[41] Moreover, chitobiose and phellodensin F showed potent antioxidant activity via free radicle scavenging properties that ultimately reflected on its ability to ameliorate liver damage.[39,42] Besides, eugenitin, a phenolic metabolite, was previously recorded to possess notable antioxidant and anti-inflammatory activity that in turn could participate in the liver protective activity.[43] Thus, it can be concluded that the *M. polymorpha* showed marked hepatoprotective activity in a dose-dependent manner that further consolidated its traditional use.

CONCLUSIONS

According to the current findings, *M. polymorpha* showed marked hepatoprotective activity counteracting hepatocellular injury in paracetamol-treated mice in a dose-dependent manner. This was evidenced by the amelioration of liver stress markers as AST, ALT, ALP, and TPs in addition to normalization of antioxidant parameters such as LPO, SOD,

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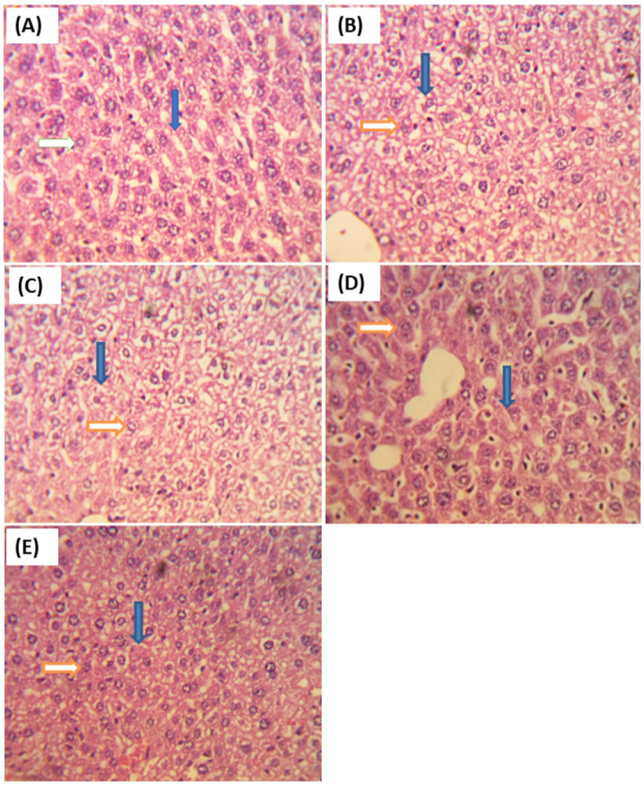

Figure 5. Histopathology of paracetamol intoxicated mice liver sections treated with different hepatoprotective agents 40×; (A) control group; (B) paracetamol intoxicated rats; (C) silymarin-treated group; (D) MP-250-treated group; and (E) MP-500-treated group.

and GPx. This was also accompanied by adjusting the lipid profile such as TB, TC, TG, LDL, VLDL, TL, and CH/HDL and elevating HDL that was further supported by the histopathological examination of the dissected liver sections. Furthermore, UHPLC−QTOF−MS metabolic profiling of the chloroform extract of *M. polymorpha* L. led to the tentative identification of 28 compounds belonging to various classes including phenols, quinolones, phenylpropanoid, acylaminosugars, terpenoids, lipids, and fatty acids to which *M. polymorpha* hepatoprotective activity is attributed. Thus, it can be concluded that this plant could serve as a valuable choice for the treatment of hepatotoxicity that further consolidated its traditional use. However, further preclinical studies are highly recommended to be conducted to further ascertain the obtained results.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01867.

> Effect of *Marchantia polymorpha* at doses of 250 and 500 mg/kg and silymarin (50 mg/kg) on liver biomarkers in mice and effect of *M. polymorpha* at doses of 250 and 500 mg/kg and silymarin (50 mg/kg) on the lipid profile in mice (PDF)

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Notes

The authors declare no competing financial interest.

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