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In vivo HEPATOPROTECTIVE ACTIVITY OF *Pyrrosia heterophylla* (L.) M.G. PRICE, A FERN USED IN INDIAN TRADITIONAL MEDICINE

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pyrrosia heterophylla is an epiphytic fern used in Indian tribal and traditional medicine for treatment of jaundice and some inflammatory conditions such as asthma, swellings and pain. The aim of the present study was to evaluate the hepatoprotective effect of different leaf extracts of *P. heterophylla* on acetaminophen-intoxicated male Wistar rats. The hepatoprotective effect of ethyl acetate and methanol extracts of *P. heterophylla* (PHE and PHM respectively) was evaluated by studying changes in liver function markers in serum such as AST, ALT, alkaline phosphatase, gamma-glutamyl transferase and bilirubin. *In vivo* antioxidant effect of the extracts was evaluated by analysing the lipid peroxidation levels and antioxidant systems in liver. Histopathological study of liver tissue was done to support the biochemical analyses. Both PHE and PHM were found to have significant hepatoprotective effect on acetaminophen intoxicated Wistar rats in a dose dependent manner. Serum markers of hepatotoxicity as well as tissue lipid peroxidative products were maintained similar to normal in the rats pre-treated with extracts. Biochemical data was supported by results of the histological studies. The study substantiated the traditional use of *P. heterophylla* as a hepatoprotective agent. Leaf extracts of *P. heterophylla* were able to provide significant hepatoprotection in acetaminophen treated rats with PHE being more effective than PHM.

Keywords: *Pyrrosia heterophylla*; hepatoprotective; antioxidant; acetaminophen.

1. INTRODUCTION

The liver is the major site for biotransformation of xenobiotics and it helps to provide protection against toxic substances that enter human body. However, the biotransformation of some of the xenobiotics results in the production of more toxic metabolites the exposure to which results in damage of liver cells and

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subsequent pathologic conditions [1]. Drug-induced liver injury (DILI) is the hepatotoxicity induced by drugs and presents a broad spectrum of symptoms. Hepatic cell death following drug intake is the most common manifestation. Acetaminophen (APAP) induced hepatotoxicity is a typical example of predictable DILI that occurs in a dose dependent manner [2].

APAP is the most widely used over-the-counter analgesic and antipyretic. It is safe at the therapeutic doses but overdose can cause severe hepatic injury [3]. Drug metabolizing enzymes in the liver convert APAP into a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) and this is the major initiator of toxicity [4, 5]. NAPQI, at non-toxic doses, is efficiently detoxified by glutathione [6, 7]. But after the intake of overdoses of APAP, the glutathione stores were found to be depleted by as much as 80-90 % [4, 8] as a result of which the NAPQI bind covalently to cysteine groups on proteins. The primary targets of covalent binding of NAPQI are mitochondrial proteins as well as proteins involved in cellular ion control [9] and plasma membrane ATPase [10]. This results in subsequent loss of energy production, activity and function of critical cellular proteins leading to cell death and lysis. Increased oxidative stress as well as inflammatory mechanisms of the innate immunity were also found to be linked with acetaminophen induced hepatotoxicity. Activation of Kupffer cells [11] and increased levels of proinflammatory cytokines [12] have been associated with APAP toxicity.

Use of herbal drugs for the management of liver diseases has a long history in Indian traditional and tribal systems of medicine. It is estimated that about more than 700 mono and poly herbal formulations are presently in use all over the world, for the various liver disorders [13]. For the treatment and prevention of drug induced liver toxicity, herbal formulations with antioxidant properties are proved to have greater efficiency. Several studies have shown that natural products containing flavonoids, triterpenes or polyphenols are powerful hepatoprotective agents [14, 15].

Pyrrosia heterophylla (L.) M.G. Price (*P. heterophylla*) is a fern used by some tribal populations in Kerala, India for the treatment of jaundice [16]. The plant has a long history of being used in traditional medicine as a treatment of various inflammatory conditions like asthma, swellings, pain and sprains [17]. Scientific studies validating the ethnomedicinal use of this plant are lacking. *In vitro* antioxidant screening and anti-inflammatory activity evaluation of the plant was done previously during our study which yielded positive results [18,19]. On this background, it was essential to evaluate the hepatoprotective property of *P. heterophylla*, and thereby ascertaining its ethnomedical use as a hepatoprotective agent. We have tested two different extracts of the plant against the most commonly used model of drug induced liver injury, acetaminophen induced hepatotoxicity.

2. MATERIALS AND METHODS

2.1 Animals

Young adult male rats of Wistar strain weighing 180-200 g were used for the studies on acetaminophen induced acute hepatotoxicity. The rats were kept in polypropylene cages and maintained at 25 ± 2^{o}C under 12 hour light/dark cycle. They were fed with standard rat pellet (Sai Durga Feeds, Bangalore, India). Water was provided *ad libitum*. The animals were acclimatized for a week with the laboratory conditions before starting experiments.

2.2 Chemicals

Acetaminophen was purchased from Sigma-Aldrich, India. Silymarin was purchased from Micro Labs Limited, Bengaluru, India. Kits for serum analysis were obtained from Agappe Diagnostics Ltd., India. All other chemicals and solvents used were of analytical grade and were purchased from Sigma, USA; HiMedia, Mumbai, India and Sisco Research Laboratories (SRL), India.

2.3 Preparation of Plant Extracts

Fresh whole *P. heterophylla* was collected from Thiruvananthapuram district of Kerala, India and validated with the help of experts at the Department of Botany, University of Kerala. A voucher specimen (No. KUBH 5926) was deposited at the herbarium of Department of Botany, University of Kerala. Washed the leaves in distilled water and dried in hot air oven at 40^{o}C. Powdered leaves were extracted in a Soxhlet apparatus with ethyl acetate and methanol. The temperature was never allowed to exceed the boiling point of the solvent. The temperatures were 77^{o} C and 64^{o} C for ethyl acetate and methanol respectively. Each of the extracts (PHE – ethyl acetate extract and PHM – methanol extract) were concentrated in rotary evaporator and stored below 0^{o}C till further use.

2.4 Induction of Acute Hepatotoxicity in Rats by Acetaminophen

Hepatotoxicity was induced in male Wistar rats (fasted overnight) by a single oral administration of

APAP at a dose of 3g/kg body weight, as dissolved in normal saline [20].

2.5 Design of *in vivo* Hepatoprotective Study

The present study followed the protocol of Kumar et al. [20] for evaluating hepatoprotective activity of plant extracts *in vivo*. Acclimatized animals were divided into 7 groups of 6 individuals each. The groups and the treatment protocol were as follows:

Group I : Normal (treated with vehicle, 0.1% DMSO)

Group II : Control (APAP in normal saline 3 g/kg body weight)

Group III: Standard (Silymarin 40 mg/kg + APAP)

Group IV: PHE 200 (ethyl acetate extract 200 mg/kg + APAP)

Group V : PHE 400 (ethyl acetate extract 400 mg/kg + APAP)

Group VI: PHM 200 (methanol extract 200 mg/kg + APAP)

Group VII : PHM 400 (methanol extract 400 mg/kg + APAP)

Group I and II received the vehicle once daily for 10 days in succession. Group III was standard reference group that received Silymarin at a dose of 40 mg/kg daily for 10 days. Groups IV to VII served as the experimental groups and received the respective extracts of *P. heterophylla* as described above, once daily for 10 days. Groups II to VII received APAP at a dose of 3 g/kg body weight as a single dose on 10^{th} day, one hour after the administration of vehicle (Group II), Silymarin (Group III) or the extracts (Groups IV to VII).

After 24 hours of APAP administration, rats of all groups were sacrificed by cervical dislocation and the blood was collected from the jugular vein. The liver was excised, cleaned and used for biochemical and histological studies.

2.6 Estimation of *in vivo* Antioxidant Activity

Liver tissue was used for estimations of antioxidant enzymes like SOD [21], catalase [22], glutathione peroxidase (GPx) [23] and glutathione transferase (GST) [24]. Tissue GSH levels [25] and lipid peroxides like thiobarbituric acid reactive substances (TBARS) [26] and conjugated dienes (CD) [27] were also estimated. Protein concentration in tissue homogenates was determined [28] for calculating enzyme activities.

2.7 Estimation of the Serum Biochemical Markers of Hepatotoxicity

Serum was collected and used for the estimation of liver function markers. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT) and lactate dehydrogenase (LDH) were determined using Liquicheck kits from Agappe Diagnostics, India, as per the manufacturer's instructions. Serum albumin, total protein and bilirubin concentration in serum were also estimated using the kits from Agappe Diagnostics, India, as per the manufacturer's instructions.

2.8 Calculation of Percentage of Hepatoprotection

Hepatoprotective activity of the extracts and the standard drug (silymarin) was calculated using the following equation [29].

$$Hepatoprotective\ activity\ (\%) = 1 - \frac{[PA - N]}{[A - N]} \times 100$$

Where, PA - measured variables in rats treated with plant extracts or silymarin + APAP (test).
N - measured variables in rats treated with vehicle alone (normal).
A - measured variables in rats treated with vehicle + APAP (control).

2.9 Histopathological Examination

A portion of liver from the treated animals was used for evaluation of histopathological changes. Pieces of cleaned liver tissue were fixed in neutral buffered formalin for 48 hours. After fixation, tissues were dehydrated through a series of graded ethanol. Following dehydration and clearing (in xylene), tissues were kept for infiltration with paraffin wax at 65^{o}C. Paraffin blocks with embedded tissues were prepared and sectioned with the help of microtome (Leica RM2125). 5 µm thick sections were dewaxed, rehydrated and stained using haematoxyline-eosin (H&E) staining. Stained sections were mounted on microscopic slides and observed for any histopathological manifestations.

2.10 Statistical Analysis

Data obtained were analyzed using one way ANOVA followed by Duncan's multiple range test. Difference between means at $p < 0.01$ was considered significant.

3. RESULTS

3.1 Effect of PHE and PHM on Antioxidant System in the Liver of Acetaminophen Intoxicated Rats

The activity of tissue antioxidant enzymes namely SOD, CAT, GPx and GST were found to be decreased significantly ($p < 0.01$) in the liver of APAP intoxicated rats compared to the control group (Table 1). Pre-treatment of the animals with PHE and PHM was found to prevent the lowering of antioxidant enzyme levels in liver. The ethyl acetate extract at a dose of 400 mg/kg could ensure ~ 90 % hepatoprotection in terms of CAT, GPx and GST activities whereas in the case of SOD, it could preserve 76.38 % of activity compared to normal group (Fig. 1). Methanol extract was able to protect ~ 90 % activity of GPx and significantly preserve the function of other antioxidant enzymes as well. Acetaminophen treatment significantly reduced the GSH stores ($p < 0.01$) in the liver tissue of rats (Table 1). Pretreatment with PHE and PHM was effective in preventing the depletion of GSH in liver cells under APAP toxicity. Significant increase ($p < 0.01$) in the GSH levels was observed in plant extract treated groups at a dose of 400 mg/kg, compared to the control.

3.2 Effect of PHE and PHM on Lipid Peroxidation in the Liver of Acetaminophen Intoxicated Rats

The products of lipid peroxidation namely, TBARS and CD were significantly increased ($p < 0.01$) in the liver of rats treated with acetaminophen compared to normal group (Table 2). The concentration of lipid peroxidation products in liver of rats pre-treated with

Table 1. Effect of different extracts of *P. heterophylla* on antioxidant enzymes and GSH in liver of acetaminophen intoxicated rats

Group	SOD (U/mg protein)	Catalase (U/mg protein)	GPx (U/mg protein)	GST (U/mg protein)	GSH (µM/g tissue)
Normal	1.78 ± 0.03	70.91 ± 0.87	163.67 ± 4.01	0.682 ± 0.015	23.05 ± 0.15
Control (APAP)	0.27 ± 0.01 †	34.07 ± 1.86 †	125.83 ± 7.05 †	0.273± 0.013 †	9.54 ± 0.09 †
APAP + Silymarin	1.46 ± 0.05 †*	66.00 ± 1.34 †*	160.33 ± 0.61 *	0.643 ± 0.018 *	22.19 ± 0.27 †*
APAP + PHE 200	0.70 ± 0.02 †*	45.93 ± 1.47 †*	131.00 ± 1.86 †	0.403 ± 0.011 †*	13.25 ± 0.17 †*
APAP + PHE 400	1.42 ± 0.02 †*	67.36 ± 1.06 *	160.00 ± 2.03 *	0.643 ± 0.019 *	20.59 ± 0.16 †*
APAP + PHM 200	0.60 ± 0.02 †*	40.43 ± 0.99 †*	129.17 ± 2.39 †	0.327 ± 0.018 †*	13.63 ± 0.18 †*
APAP + PHM 400	0.91 ± 0.01 †*	56.82 ± 1.53 †*	159.83 ± 2.02 *	0.568 ± 0.018 †*	17.93 ± 0.18 †*

*Values expressed as mean ± SEM of 6 animals in each group. † significantly different from normal; * significantly different from control, $p < 0.01$*

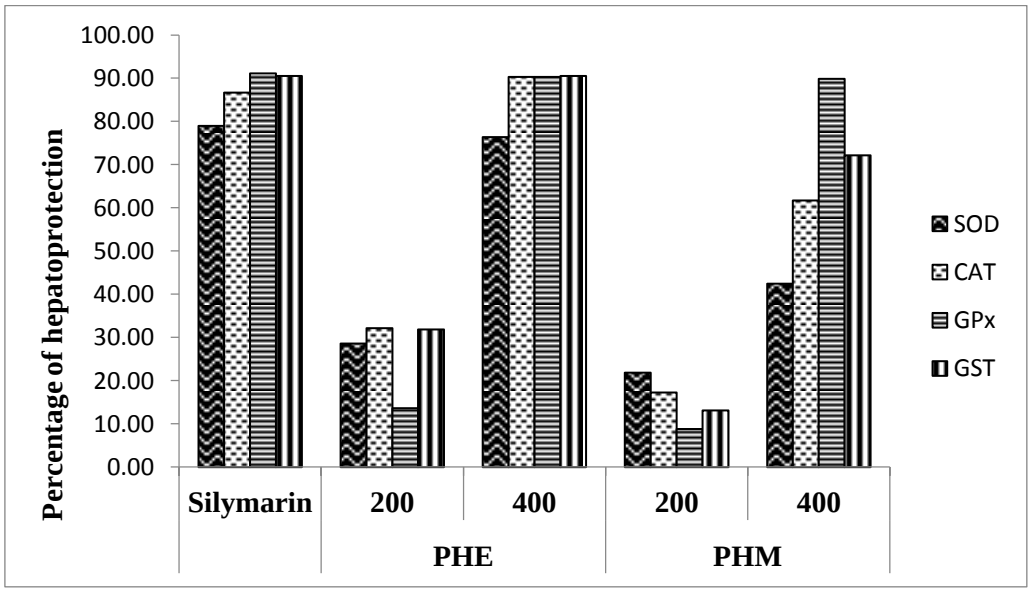

Fig. 1. Percentage of hepatoprotection by ethyl acetate and methanol extracts of *P. heterophylla* with respect to antioxidant enzymes in liver of acetaminophen intoxicated rats

PHE and PHM was significantly lower ($p < 0.01$) than the control group. It was evident that the extracts could effectively prevent the oxidative damage induced by APAP in liver cell membranes.

Table 2. Effect of different extracts of *P. heterophylla* on lipid peroxidation in liver of acetaminophen intoxicated rats

Group	TBARS (µM/g tissue)	CD (µM/g tissue)
Normal	0.02 ± 0.00	2.33 ± 0.06
Control	0.17 ± 0.02 †	10.67 ± 0.11 †
Standard	0.02 ± 0.00*	2.73 ± 0.14 †*
PHE 200	0.06 ± 0.00 †*	6.91 ± 0.04 †*
PHE 400	0.02 ± 0.00 *	2.82 ± 0.08 †*
PHM 200	0.06 ± 0.00 †*	7.00 ± 0.02 †*
PHM 400	0.03 ± 0.00 *	3.87 ± 0.10 †*

*Values expressed as mean ± SEM of 6 animals in each group. † Significantly different from normal, * significantly different from control, $p < 0.01$*

3.3 Effect of PHE and PHM on Liver Function Marker Enzymes of Acetaminophen Intoxicated Rats

Activity of the enzymes AST, ALT, ALP, GGT and LDH after 24 hours of acute intoxication with acetaminophen are given in Table 3. The results indicated that the concentration of the liver function markers was significantly elevated ($p < 0.01$) in animals intoxicated with acetaminophen. It was evident that APAP toxicity caused the leakage of hepatic enzymes from the damaged hepatocytes. Treatment with PHE and PHM significantly reduced the concentration of these enzymes in serum which proved that the extracts helped in retaining the hepatocellular integrity. The effect was dose dependent with maximum hepatoprotection in groups treated with 400 mg/kg of the extracts. The percentage of hepatoprotection offered by the two extracts as compared to the standard drug silymarin is given in Fig. 2. PHE was found provide hepatoprotection similar to that of standard drug silymarin. PHM was also found to be significantly protective against APAP induced toxicity in the cells.

3.4 Effect of PHE and PHM on Protein and Bilirubin Levels in Serum of Acetaminophen Intoxicated Rats

Total protein and albumin levels in serum of rats were markedly decreased ($p<0.01$) and the bilirubin level was significantly elevated ($p<0.01$) by acetaminophen intoxication (Table 4). The deranged protein and bilirubin metabolism in liver was ameliorated by the plant extracts. In rats that received PHE and PHM at a dose of 400 mg/kg body weight, the total protein, albumin and bilirubin levels were maintained at concentrations near normal values.

Table 3. Effect of different extracts of *P. heterophylla* on hepatic function marker enzymes in serum of acetaminophen intoxicated rats

Group	AST (U/L serum)	ALT (U/L serum)	ALP (U/L serum)	GGT (U/L serum)	LDH (U/L serum)
Normal	81.83 ± 1.80	39.00 ± 1.24	111.83 ± 2.23	8.73 ± 0.19	167.50 ± 0.92
Control (APAP)	165.83 ± 1.64 †	108.50 ± 2.68 †	447.50 ± 1.38 †	14.50 ± 0.34 †	755.33 ± 1.52 †
APAP + Silymarin	82.00 ± 1.46 *	43.67 ± 0.80 †*	116.33 ± 1.80 *	9.17 ± 0.31 *	179.33 ± 0.71†*
APAP + PHE 200	117.33 ± 1.09 †*	81.67 ± 0.99 †*	358.17 ± 0.79 †*	11.67 ± 0.33 †*	585.83 ± 1.01† *
APAP + PHE 400	85.00 ± 1.06 †*	47.33 ± 0.95 †*	128.67 ± 1.50 †*	9.00 ± 0.26 *	181.67 ± 1.43† *
APAP + PHM 200	126.00 ± 1.93† *	92.67 ± 1.31† *	394.33 ± 1.94 †*	12.83 ± 0.60 †*	599.33 ± 1.15 †*
APAP + PHM 400	88.50 ± 1.18 †*	58.83 ± 1.40 †*	199.00 ± 2.21 †*	10.67 ± 0.33 †*	196.33 ± 1.74† *

*Values expressed as mean ± SEM of 6 animals in each group. † Significantly different from normal; * significantly different from control, $p < 0.01$*

Table 4. Effect of different extracts of *P. heterophylla* on serum protein and bilirubin concentration in acetaminophen intoxicated rats

Group	Protein		Bilirubin	
	Total protein (g/dL)	Albumin (g/dL)	Total (mg/dL)	Direct (mg/dL)
Normal	7.37 ± 0.09 *	4.10 ± 0.11 *	0.11 ± 0.01 *	0.05 ± 0.003 *
Control	5.08 ± 0.15 †	2.42 ± 0.12 †	0.75 ± 0.02 †	0.25 ± 0.020 †
Standard	7.30 ± 0.16 *	3.90 ± 0.08 *	0.11 ± 0.02 *	0.05 ± 0.004 *
PHE 200	5.72 ± 0.11 †*	2.80 ± 0.07 †	0.59 ± 0.01 †*	0.12 ± 0.01 †*
PHE 400	7.33 ± 0.08 *	4.17 ± 0.10 *	0.12 ± 0.01 *	0.06 ± 0.004 *
PHM 200	5.97 ± 0.22 †*	2.57 ± 0.14 †	0.62 ± 0.02 †*	0.18 ± 0.02 †*
PHM 400	7.02 ± 0.08 *	3.88 ± 0.05 *	0.20 ± 0.01 †*	0.08 ± 0.006 †*

*Values expressed as mean ± SEM of 6 animals in each group. † Significantly different from normal; * significantly different from control, $p < 0.01$*

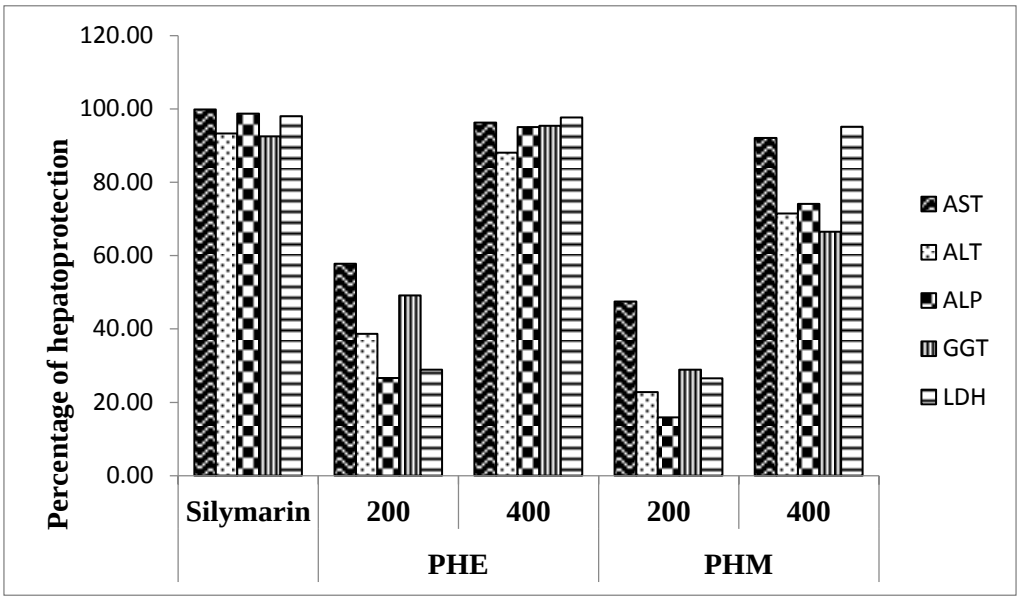

Fig. 2. Percentage of hepatoprotection by ethyl acetate and methanol extracts of *P. heterophylla* with respect to liver function markers in serum of acetaminophen intoxicated rats

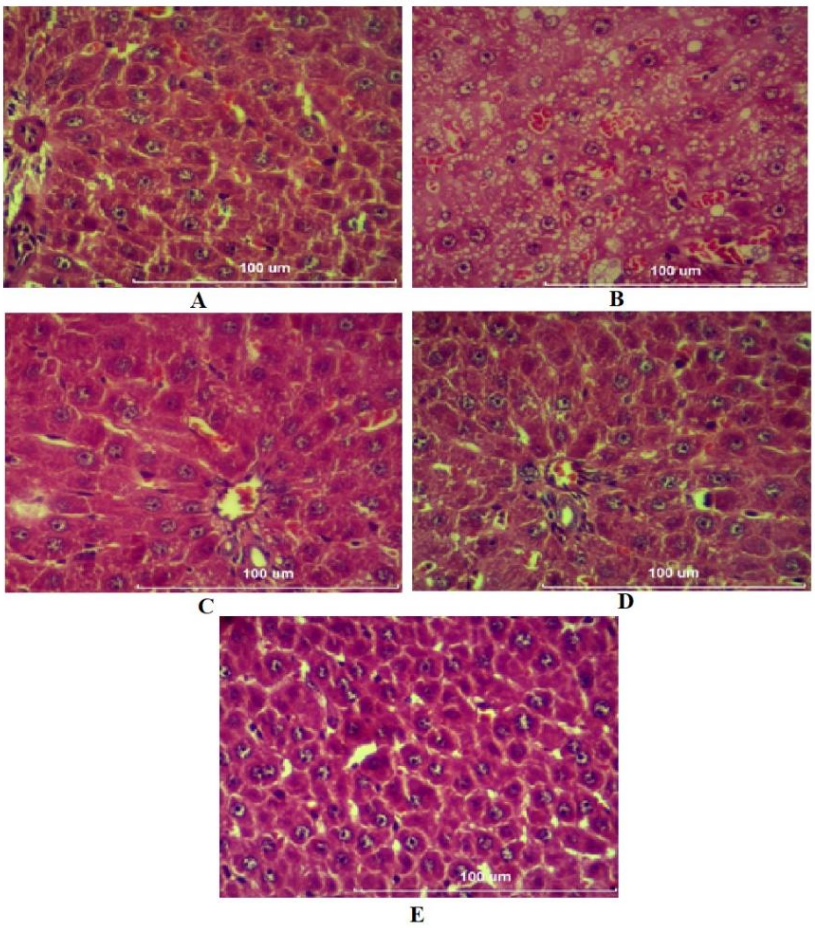

Fig. 3. Micrographs of histological sections of A. normal rats; B. APAP intoxicated control rats; C. PHE (400 mg/kg) + APAP; D. PHM (400 mg/kg) +APAP; E. Silymarin (40 mg/kg) + APAP

3.5 Histopathological Examination

Histological evaluation of liver tissue from normal animals revealed normal hepatic parenchyma with well-defined sinusoids (Fig. 3). The cells showed normal cytoplasm, well preserved prominent nuclei, cell membrane, etc. Liver sections of acetaminophen treated control rats showed characteristic symptoms of toxicity, namely, centrilobular necrosis, collapsed sinusoids, microvesicular steatosis (small fat filled vesicles), etc. Histological examination of liver from rats treated with 400 mg /kg of PHE and PHM showed normal hepatocytes with well-preserved sinusoids. No sign of micro or macro vesiculation or necrosis was observed in these tissues. Liver of rats treated with Silymarin also revealed normal hepatic architecture. The tested extracts of *P. heterophylla* were evidently protective against APAP induced cellular damage in liver.

4. DISCUSSION

Results of various analyses done in the present study helped us to scientifically validate the ethnomedical use of *P. heterophylla* as a hepatoprotective fern. During the metabolism of APAP at therapeutic doses, it is channelled through 3 pathways in the liver. About 90 percentage of the absorbed APAP is passed through the phase II metabolic pathways. In this, APAP is conjugated by UDP-glucuronosyl transferases (UGT) and sulfotransferase (SULT) to form glucouronidated and sulfated metabolites. They can be easily removed in urine [28]. About 2% of APAP is excreted through urine unchanged. 8-10 % of APAP is funneled through the cytochrome P450 (CYP) pathways. This results in the production of the highly toxic reactive metabolite N-acetyl-para-benzo-quinone imine (NAPQI) [5,30], which is the compound responsible for APAP-induced hepatotoxicity. After a highly toxic overdose of APAP, the sulfation and glucuronidation pathways get saturated and higher proportions of the drug are converted to NAPQI. Detoxification of NAPQI in liver is done mainly by conjugation with GSH. But at toxic doses, increased concentration of NAPQI tends to deplete the GSH stores which makes it to form adducts with cellular proteins [8]. The primary targets of NAPQI are mitochondrial and ion channel proteins, thus disrupting the energy production and ion balance of the cells. This results in mitochondrial permeability transition and necrotic cell death [31,32,33]. Glutathione depletion also leads to increased oxidative stress in the cells via a Fenton mechanism in which ferrous ions reduce the peroxides forming a highly reactive hydroxyl radical. This hydroxyl radical can initiate a chain reaction of lipid peroxidation leading to oxidation of membrane lipids, nucleic acids and proteins [34]. Due to the lipid peroxidation reactions the membrane integrity of hepatocytes is lost and this results in the leakage of hepatic enzymes into the serum. In the present study the entire hepatic marker enzyme levels in serum were found to be increased significantly in animals treated with acetaminophen alone, compared to the normal group.

Antioxidant enzyme status and concentration of lipid peroxidation products in liver showed that APAP has induced significant oxidative stress in liver which has led to lipid peroxidative damage of hepatocyte membranes. Histopathological studies also supported this finding. Liver tissues of APAP intoxicated rats showed significant damage to hepatocyte membrane and collapsed sinusoidal spaces. From the results we could infer that APAP toxicity induced oxidative damage to cell membranes which ultimately resulted in leakage of hepatic function marker enzymes such as AST, ALT, etc. Increased level of serum bilirubin was also indicative of hepatocellular damage and deranged bilirubin metabolism in liver. Protein synthetic machinery in hepatocytes was disrupted as evident from the lower levels of total protein and albumin in serum of APAP intoxicated rats.

Administration of PHE and PHM was found to be highly protective against the acetaminophen induced toxicity in liver. In the present study, when rats were pre-treated with PHE and PHM they could prevent APAP induced damage in a dose dependent manner. This is due to the strong antioxidant and anti-inflammatory property of these extracts which was proved during our earlier studies [18,19]. GC-MS analysis of these extracts during our previous studies also confirmed the presence of compound such as eugenol, 2,3,dihydro-dihydrobenzofuran, 2-Methoxy-4-vinylphenol, l-(+)-Ascorbic acid 2,6-dihexadecanoate, phytol, etc. in PHE and l-(+)-Ascorbic acid 2,6-dihexadecanoate, 4-tert-Butoxystyrene, 1-(2,5-Dihydroxyphenyl) ethanone, etc. in PHM [35]. Most of the herbal products are believed to exert their protective effects via the removal of free radicals from the cell environment and thus protecting the membrane lipids and other macromolecules from the ROS induced damage [15,36]. Eugenol has been found to exhibit very good antioxidant efficacy [37, 38] as well as hepatoprotective effect against different toxic agents [39, 40]. Many plant extracts and essential oils from plants containing phytol has been shown to exhibit antioxidant properties [41]. The antioxidant property of several other species of *Pyrrosia* have been reported earlier [42,43,44]

P. heterophylla extracts namely PHE and PHM could effectively scavenge free radicals generated during APAP metabolism which led to the prevention of lipid peroxidative damage to cells. This was in agreement with the result of our previous *in vitro* antioxidant study [33]. *P. heterophylla* extracts could prevent the leakage of hepatic enzymes such as AST, ALT, ALP, GGT and LDH. As the hepatocyte health and integrity was maintained, normal levels of bilirubin and protein was regained in rats treated with the extracts. Due to the antioxidant activity of the extracts, normal antioxidant systems of liver which includes GSH, GPx and GST was also maintained similar to normal levels. The anti-inflammatory activity of these extracts especially their ability to inhibit TNF-α has been confirmed during our earlier studies [34]. This also could have contributed to the hepatoprotective effect of the extracts.

Since the ethyl acetate and methanol extracts of *P. hetrophylla* leaves contain several compounds with known and unknown bioactivities, the synergistic action of these compounds have resulted in the prominent hepatoprotective effect against acetaminophen induced toxicity. Although both the extracts showed dose dependent hepatoprotective activity, PHE was found to offer better protection, owing to the plenitude of bioactive phytochemicals it possesses. Thus, the present *in vivo* investigation conclusively substantiates ethnomedical potential of *P. heterophylla* especially hepatoprotective functions.

5. CONCLUSION

This study presents the scientific validation of the hepatoprotective activity of a fern used in tribal and traditional systems of medicine in India. Ethyl acetate and methanol leaf extracts of the plant have the potential to preserve the antioxidant system in liver cells thereby providing protection to hepatocytes against acetaminophen induced toxicity.

ETHICAL APPROVAL

Ethical clearance was obtained from the Institutional Animal Ethical Committee (No. A1/7/2012/1396/a/10/ CPCSEA) of Mar Ivanios College (Autonomous), Thiruvananthapuram, Kerala, India where the animal experiments were performed.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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