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Preclinical study on hepatoprotective potential and antioxidant status analysis of the fruit extract of *Rivina humilis* L. (red pigeon berry) in rats

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ABSTRACT

Hepatoprotective and antioxidant potential of hydromethanolic extract of *Rivina humilis* L. fruit (HMERH) was studied using the carbon tetrachloride (CCl_4) and paracetamol (PCM) induced hepatotoxicity in Wistar rats. In both models, rats were divided into five groups having six rats in each. Group I received distilled water. Group II, disease control, Group III received 100 mg/kg of Silymarin. Group IV and Group V received 500, 1000 mg/kg of HMERH. All groups except Group II received treatment twice daily for 7 days. CCl_4 (1.5 mL/kg, PCM (2 g/kg), p.o) were administered after the last dose. The degree of liver protection against toxicity was measured by estimating serum liver marker enzymes and protein. In addition, estimation of lipid profile, liver weight, and histopathological examination were done. Peroxidase (P), Catalase, glutathione, total protein, superoxide dismutase, and malondialdehyde were also estimated from the part of liver isolated. HMERH 500 and 1000 mg/kg treated groups showed a significant ($P < 0.001$) decrease in serum marker enzymes as well as total bilirubin levelswere also restored. It also stabilize and restored the lipid profile and endogenous antioxidant enzyme system. Hence, the hepatoprotective effect of HMERH 500 and 1000 mg/kg against CCl_4 as well as PCM may possibly be mediated through antioxidant activity.

Keywords: Antioxidant, betalain, carbon tetrachloride, hepatoprotective, paracetamol, *Rivina humilis* L. fruit

INTRODUCTION

All endogenous and exogenous substances entering systemic circulation are metabolized in the liver; thereby, it facilitates removal of waste/toxins from the body. It maintains vital regulation by the production of various constituents essential for life. Hepatic injury is connected with the alteration of its functions. Vulnerable diseases such as liver cancer, hepatitis, liver cirrhosis, other hepatic disorders, and alcohol intake affect the normal liver functions. Exposure to xenobiotics, oxidative stress, environmental pollutants, acute, and chronic inflammation may also leads to liver diseases.[1] Xenobiotics such as carbon tetrachloride (CCl_4), alcohol, and acetaminophen paracetamol (PCM) are the major toxicants that damage the liver. CCl_4 is metabolized by a liver enzyme cytochrome P_{450} (CYP_{450}) and converted to trichloromethyl which act as free radicals or reactive oxygen species. This metabolite causes hepatocellular injury through lipid peroxidation result in imbalance between oxidants and the antioxidant core enzymes for defense. Similarly, PCM get converted into responsive toxic metabolite as N-acetyl-p-benzo-quinone imine by CYP_{450} which irreversibly bind with sulfhydryl (-SH) groups of glutathione (GSH) through conjugation reaction resulting in depletion of endogenous antioxidant such as GSH. Surplus generation of this metabolite causes the initial hepatic damage followed by activation of tumor necrosis factor α, an inflammatory mediator which in turn contribute to tissue necrosis. Therefore, CCl_4 and PCM induced chronic liver injury is considered as an experimental models which can be used for the evaluation of the hepatoprotective drugs.[2]

Natural products have been utilized broadly for the treatment of numerous infections and are occupied in greater part in drug discovery which may help to improve medications

in treating malignancy and liver infection among others.[3] *Rivina humilis* L. belongs to family *Phytolaccaceae*, also called as bright red pigeon berry. It is an herbaceous bushy wild plant found in shaded soils. It was traditionally used for various diseases such as gonorrhea and jaundice[4,5] but the vast literature survey fails to report the hepatoprotective effect. It contains an important phytoconstituent called betalain a natural antioxidant. It is active against oxidative stress related diseases such as inflammations, atherosclerosis, ischemia, asthma, diabetes, cardiovascular diseases, and viral infections.[6] The existing study was to screen the hepatoprotective potential of *R. humilis* L. and was conducted on CCl_4 and PCM-induced hepatotoxicity in rats by using hydromethanolic extract of *R. humilis* fruit (HMERH).

MATERIALS AND METHODS

Plant Materials

Fruits of *R. humilis* L, were collected in the month of October 2020 from Marthandam, Tamil Nadu and Kayamkulam, Kerala, India. The collected whole plant material was identified and authenticated by Professor Dr. Sandhya P, HOD, Department of Botany, NSS College, Pandalam. The specimen of herbarium BOHDOS-2/27/11/2020 was deposited at the Botany Department, NSS College, Pandalam, Kerala.

Preparation of the Fruit Extract

The dried powdered fruits material of *R. humilis* was extracted by cold maceration using water and methanol (70:30) for a period of 72 h with occasional shaking till completion of extraction. After 72 h, the mixture was strained through muslin cloth and squeezed to remove all the remaining liquid. Solvent was recovered using rota evaporator under reduced pressure. The crude extract was named as HMERH, and used for this experimental study.

Preliminary Phytochemical Analysis

A preliminary phytochemical study was conducted on the HMERH fruit to analyze various phytoconstituents.[7]

Experimental Animal

Wistar albino rats (200–230 g) of both genders were chosen. They were accustomed under standard research center ecological conditions for a week before the experiment. Taken care of all the animals with standard rat feed and water *ad libitum*. Use of animals was approved by the Institutional Animal Ethics Committee of St. Joseph's College of Pharmacy, Chrerthala, Kerala (Protocol No.: SJCP/IEC/2020/12/15).

CCl_4 Induced Hepatotoxicity

Thirty rats (200–230 g, both genders) were chosen and separated into seven groups of six rats in each, and the treatment method as followed as that of Asirvatham and Akhil 2020.[8] Group I received 1 mL of distilled water (vehicle) orally 2 times in a day for 7 days. Group II animals received one mL of distilled water orally, twice a day for 7 days and one mL of 1:1 ratio of 1.5 mL/kg of CCl_4 and olive oil, administered orally on the 7th day. Group III received 100 mg/kg of Silymarin p.o twicea day for 7 days and 1 mL of 1:1 ratio of 1.5 mL/kg of CCl_4 and olive oil, administered orally on the 7th day. Group IV received 500 mg/kg of HMERH p.o twice a day for 7 days and 1 mL of 1:1 ratio of 1.5 mL/kg of CCl_4 and olive oil administered orally on the 7th day. Group V received 1000 mg/kg of HMERH p.o twice a day for 7 days and 1 mL of 1:1 ratio of 1.5 mL/kg of CCl_4 and olive oil administered orally on the 7th day. On the 7th day, 18 h post-administration of CCl_4 all the animals from groups 1–5 were weighed, sacrificed by euthanasia, and blood was collected immediately for measurement of serum parameters. Livers were isolated and weighed. A part of the livers was used for histopathological study and another part for estimation of tissue antioxidant enzymes such as peroxidase (P), catalase (CAT), GSH, total protein (TP), superoxide dismutase (SOD), and malondialdehyde (MDA).[9]

PCM-induced Hepatotoxicity

Thirty rats (200–230 g, both genders) were chosen and separated into seven groups of six rats in each and the treatment method followed was as that of Asirvatham and Akhil 2020.[8] Group I received 1 mL distilled water (vehicle) orally, 2 times in a day for 7 days. Group II animals received one mL of distilled water 2 times in a day, orally for 7 days and 1 mL of PCM (2 g/kg) administered orally on the 7th day. Group III received 100 mg/kg of Silymarin p.o 2 times in a day, orally for 7 days and 1 mL of PCM (2 g/kg) administered orally on the 7th day. Group IV received 500 mg/kg of HMERH p.o 2 times in a day, orally for 7 days and 1 mL of PCM (2 g/kg) administered orally on the 7th day. Group V received 1000 mg/kg of HMERH p.o 2 times in a day, orally for 7 days and 1 mL of PCM (2 g/kg) administered orally on the 7th day. On 7th day, 18 h post-administration of PCM all the animals from groups 1–5 were weighed, sacrificed by euthanasia and blood was collected immediately for measurement of serum parameters. Livers were isolated and weighed. A part of the livers was used for histopathological study and another part for estimation of tissue antioxidant enzymes such as peroxidase (P), CAT, GSH, TP, SOD, and MDA.[9]

Estimation of Liver Enzyme Markers and Proteins

The weight of the liver was expressed in g units. Analytical kits and procedure manual from Agappe Diagnostics Ltd., Kerala, India, were required for the estimation of serum marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) using an auto analyzer from Roche, USA.

Estimation of Lipid Profile

Lipid profile including low density lipoprotein (LDL), triglycerides (TG), high density lipoprotein (HDL), and total cholesterol (TC) were estimated by auto analyzer using reagent kit.[2]

Estimation of Tissue Antioxidant Enzymes

The tissue homogenate of liver was prepared as follow; liver homogenate (10% w/v) was prepared in KCl buffer (0.15 M) then centrifuged for 10 min at 8000 rpm. The supernatant

(upper liquid layer) was separated and was used for the assessment of peroxidase (P), CAT, GSH, TP, SOD, and MDA. One more part of the tissue homogenate also prepared at a concentration of 10% w/v with 5 M, pH 7.4 sucrose phosphate buffer (0.25% w/v). This was also centrifuged at 8000 rpm for 10 min to remove the tissue debris, and the clear supernatant fluid was taken for the assessment of GSH and SOD. All the estimations were done with reagent kit.[9]

Statistical Analysis

Data were expressed as mean ± standard error of the mean; one-way analysis of variance followed by Students-Newman-Keuls comparison test. Statistically significant differences; where $P < 0.05$ was considered significant.

RESULTS

Phytochemical analysis of HMERH was conducted for identification of various phytoconstituents such as alkaloids, steroids, flavonoids, saponins, tannins, terpenoids, glycosides, proteins, carbohydrates, and phenols. The results were shown in Table 1. HMERH showed the existence of steroids, alkaloids, phenols. flavonoids, terpenoids, glycosides, saponins, carbohydrates, and tannins.

Liver enzymes and protein levels in serum were estimated and shown in Table 2. Increased level of AST, ALT, ALP, and total bilirubin (TB) were found with CCl_4 alone treated rats. The elevated liver enzymes were significantly ($P < 0.001$) normalized in HMERH treated rats. The TP and TA levels were reduced in disease control rats and significantly stabilized in 500 and 1000 mg/kg doses of HMERH. The 500 mg/kg of HMERH revealed less significant ($P < 0.01$) effects than the higher dose on liver marker enzymes ALT and ALP.

Table 1: Preliminary phytochemical analysis of HMERH

S. No.	Constituents	HMERH
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Steroids	+
5	Glycosides	+
6	Saponins	+
7	Terpenoids	+
8	Carbohydrates	+
9	Proteins	-
10	Phenol	+

+: Present, -: Absent, HMERH: Hydromethanolic extract of *Rivina humilis*

Change in lipid profile and liver weight of CCl_4-induced hepatotoxicity in rats was shown in Table 3. The LDL, TC, and TG levels were significantly ($P < 0.001$) elevated in the disease control group but decreased in the 500 and 1000 mg/kg HMERH treated groups. The extract also raised the HDL cholesterol level to normal, whereas it was decreased in CCl_4 alone treated group. Only rats treated with CCl_4 (1.5 mL/kg) showed a significant ($P < 0.001$) increase in liver weight, whereas rats which were pretreated with 500 and 1000 mg/kg HMERH and standard Sylimarin showed no significant changes.

The effect of 500 and 1000 mg/kg HMERH on liver antioxidant enzymes level in CCl_4 induced hepatotoxicity in rats was shown in Table 4. Significantly ($P < 0.001$) high level of MDA was found with only CCl_4 (1.5 mL/kg) treated rats. It was found to be less in normal as well as extract treated groups of animals. Other antioxidants enzymes were significantly ($P < 0.001$) reduced in only CCl_4 treated rats, whereas pretreatment with 500 and 1000 mg/kg HMERH had significantly increased these free radical defense enzymes and reduced MDA levels.

The results of liver enzymes and protein levels in serum from PCM-induced hepatotoxicity model were illustrated in Table 5 where an augmented level of AST, ALT, ALP, TB and reduced level of TP and TA were found with only PCM treated rats, but they were reduced significantly ($P < 0.001$) in HMERH treated rats. These enzymes were significantly stabilized and restored by continuous 7-day pretreatment with 500 and 1000 mg/kg doses of HMERH fruits. The lower dose of HMERH fruits showed fewer effects on the restoration of liver marker enzymes ALT and ALP than the higher dose.

The change in lipid profile and liver weight was examined in PCM-induced hepatotoxicity and was shown in Table 6. Except HDL, other parameters were significantly ($P < 0.001$) elevated in only PCM treated group but they were brought back to normal in the 500 and 1000 mg/kg HMERH fruits pre-treated group. The extract also raised the HDL level within normal range, it was decreased in the PCM alone treated group. The liver weight of rats which received only PCM (2 g/kg), increased significantly ($P < 0.001$) but rats which were pretreated with 500 and 1000 mg/kg of HMERH fruits and standard Sylimarin did not show any notable changes in liver weight.

Effect of 500 and 1000 mg/kg of HMERH fruits on liver antioxidant enzymes level in PCM-induced hepatotoxicity in

Table 2: Effects of HMERH on biochemical parameters in CCl_4-induced hepatotoxicity in rats

Treatment	Dose (mg/kg)	AST (U/mL)	ALT (U/mL)	ALP (U/mL)	TB (g/dL)	TP (g/dL)	TA (g/dL)
Normal	-	180.5±0.8	85.7 ±0.4	42.2±0.2	0.55±0.01	21.8±0.4	5.6±0.5
CCl_4	1.5 mL/kg	271.7±1.3	118.5±0.2	63.7±0.6	3.8±0.2	10.4±0.7	2.3±0.01
Silymarin	100 mg/kg	188.3±1.1	88.4±0.7	44.1±0.7	0.84±0.04	19.8±0.8	4.9±0.4
HMERH	500 mg/kg	204.8±1.8[a]	91.3±1.4[b]	48.6±0.4[b]	1.9±0.7[a]	18.5±0.1[a]	3.1±0.8[a]
	1000 mg/kg	190.4±1.6[a]	87.7±0.6[a]	44.1±0.8[a]	1.0±0.05[a]	20.3±0.3[a]	5.1±0.2[a]

Data expressed as Mean±SEM for six observations $P<0.001$ compared to respective CCl_4 induced group. The data were analyzed by one way ANOVA followed by students newman keuls: [a]$P<0.001$, [b]$P<0.01$. HMERH: Hydromethanolic extract *of R. humilis* fruits

Table 3: Effects of HMERH on lipid profile and liver weight of CCl_4-induced hepatotoxicity in rats

Treatment	Dose (mg/kg)	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	Liver weight (g)
Normal	-	71±1.2	78±2.3	32±1.1	88±2.3	5.6±0.4
CCl_4	1.5 mL/kg	155±1.6	162±1.8	21±1.5	203±1.8	8.2±1.2
Silymarin	100 mg/kg	82±1.1	86±1.2	29±0.5	95±2.4	5.5±0.8
HMERH	500 mg/kg	95±1.3[a]	88±0.9 [a]	28±1.9[b]	102±1.6[a]	6.1±0.2[a]
	1000 mg/kg	75±1.8[a]	80±1.4[a]	30±0.8[a]	93±1.4[a]	5.8±0.1[a]

Data expressed as Mean±SEM for six observations $P<0.001$ compared to respective CCl_4 induced group. The data were analyzed by one-way ANOVA followed by students newman keuls: [a]$P<0.001$. HMERH: Hydromethanolic extract *of R. humilis* fruits

Table 4: Effects of HMERH on antioxidant parameters in CCl_4-induced hepatotoxicity in rats

Treatment	Dose (mg/kg)	TP mg/dL	CAT U/mg tissue	SOD U/mg tissue	P nm/100 mg tissue	GSH nm/100 mg tissue	MDA nm/g protein
Normal	-	26.6±0.4	20.±0.3	13.6±0.3	42.2±0.5	62.7±0.4	7.6±0.2
CCl_4	1.5 mL/kg	12.8±0.3	7.6±0.1	5.2±0.1	32.1±0.1	26.4±0.5	25.6±0.3
Silymarin	100 mg/kg	26.6±0.4	21.3±0.2	14.5±0.7	41.4±0.5	62.2±0.3	8.1±0.2
HMERH	500 mg/kg	26.1±0.3[a]	19.9±0.2[a]	13.1±0.1[a]	42.25±0.3[a]	60.9±0.3[a]	6.6±0.04[a]
	1000 mg/kg	28.7±0.6[a]	20.9±0.3[a]	10.5±0.01[a]	43.6±0.2[a]	64.34±0.3[a]	8.38±0.2[a]

Data expressed as Mean±SEM for six observations $P<0.001$ compared to respective CCl_4-induced group. The data were analyzed by one-way ANOVA followed by students newman keuls: [a]$P<0.001$. HMERH: Hydromethanolic extract *of R. humilis* fruits

Table 5: Effects of HMERH on biochemical parameters in PCM-induced hepatotoxicity in rats

Treatment	Dose (mg/kg)	AST (U/mL)	ALT (U/mL)	ALP (U/mL)	TB (g/dL)	TP (g/dL)	TA (g/dL)
Normal	-	180.5±0.8	85.7 ±0.4	42.2±0.2	0.55±0.01	21.8±0.4	5.6±0.5
PCM	2 g/kg	220.2±2.5	130.5±1.6	74.8±0.4	2.8±0.07	8.9±0.6	1.9±0.6
Silymarin	100 mg/kg	188.3±1.1	88.4±0.7	44.1±0.7	0.84±0.04	19.8±0.8	4.9±0.4
HMERH	500 mg/kg	195.2±1.6[b]	107.8±1.1[b]	55.2±1.2[a]	1.1±0.02[a]	16. ±0.7[a]	3.9±0.8[a]
	1000 mg/kg	186.7±2.4[a]	91.2±1.5[a]	48.5±0.6[a]	0.9±0.05[a]	20.4±0.3[a]	4.5±0.5[a]

Data expressed as Mean±SEM for six observations $P<0.001$ compared to respective PCM induced group. The data were analyzed by one-way ANOVA followed by students newman keuls: [a]$P<0.001$, [b]$P<0.01$. HMERH: Hydromethanolic extract *of R. humilis* fruits

Table 6: Effects of HMERH on lipid profile and liver weight of PCM-induced hepatotoxicity in rats

Treatment	Dose (mg/kg)	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	Liver weight (g)
Normal	-	71±1.2	78±2.3	32±1.1	88±2.3	5.6±0.4
PCM	2 g/kg	180±1.2	195±1.8	19±1.5	221±1.8	9.5±1.2
Silymarin	100 mg/kg	94±1.7	82±1.6	30±1.4	91±0.9	5.1±0.3
HMERH	500 mg/kg	105±1.8[a]	112±1.1 [a]	29±0.9[a]	132±2.2[a]	6.5±0.7[a]
	1000 mg/kg	88±1.2[a]	92±1.7[a]	30±1.9[a]	102±2.4[a]	5.5±0.5[a]

Data expressed as Mean±SEM for six observations $P<0.001$ compared to respective PCM-induced group. The data were analyzed by one-way ANOVA followed by students newman keuls: [a]$P<0.001$. HMERH: Hydromethanolic extract *of R. humilis* fruits

rats was shown in Table 7. Significantly ($P < 0.001$) high level of MDA was found with only PCM treated rats, but it was found to be less with normal as well as extract treated groups of animals. Other antioxidant enzymes were significantly ($P < 0.001$) reduced in only PCM treated rats, whereas pretreatment with 500 and 1000 mg/kg HMERH significantly raised these free radical defense enzymes more like normal rats and made a significant reduction in MDA levels.

The effect of 500 and 1000 mg/kg of HMERH fruits on the protection of the liver from the damage of CCl_4 and PCM were assessed up to the cellular level by studying histopathological observations and were illustrated in Table 8. Severe fatty change, vacuolation, mononuclear aggregation, infiltrates, and multifocal hepatocytes were found in the livers of both disease control group rats and were protected with 500 and 1000 mg/kg of HMERH fruits found to be within the normal limits of hepatocytes.

DISCUSSION

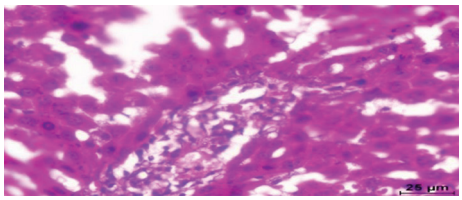
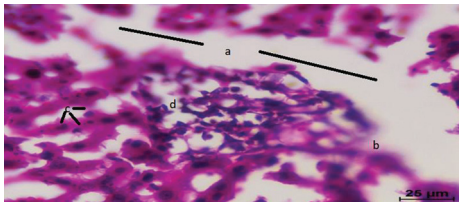
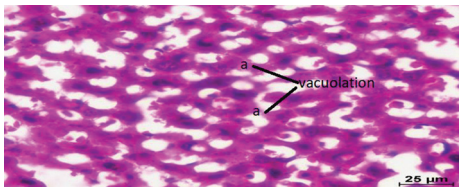
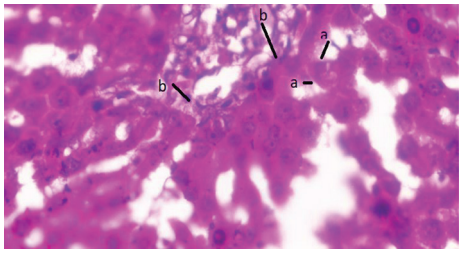
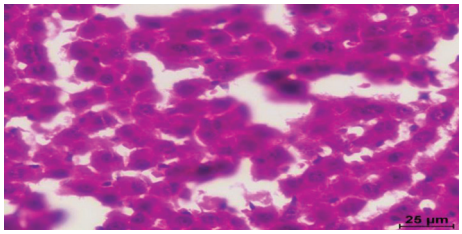
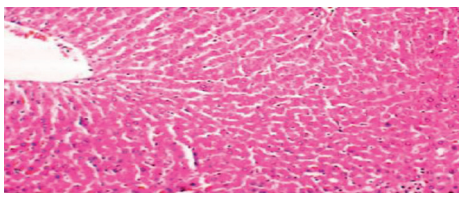
The liver is the major organ that detoxifies our blood of various harmful substances and metabolizes them in to more polar, water soluble forms to get them excreted easily. Substances such as chemotherapeutic agents, viruses, bacteria, fungus, and other agents cause various kinds of stress affecting the

Table 7: Effects of HMERH on antioxidant parameters in PCM-induced hepatotoxicity in rats

Treatment	Dose (mg/kg)	TP mg/dL	CAT U/mg tissue	SOD U/mg tissue	P nm/100mg tissue	GSH nm/100mg tissue	MDA nm/g protein
Normal	-	22.±0.3	11.5±0.1	23.6±0.4	77.5±3.2	101.9±0.6	12.9±0.7
PCM	2 g/kg	18.1±0.2	7.1±0.5	14±0.4	55.5±6.9	50.9±0.3	29.8±0.2
Silymarin	100 mg/kg	22.5±0.1	11.6±0.1	22.1±0.3	75.4±2.2	96.8±1.2	12.3±0.2
HMERH	500 mg/kg	22.3±0.9[a]	11.5±0.2[a]	20.4±0.1[a]	75.9±0.6[b]	84.6±1.5[a]	11.2±0.3[a]
	1000 mg/kg	23.3±0.5[a]	12.1±0.4[a]	24±0.5[a]	80.5±0.7[a]	96.8±0.6[a]	10.7±0.3[a]

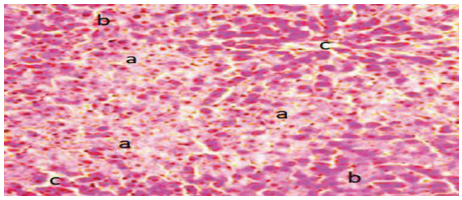
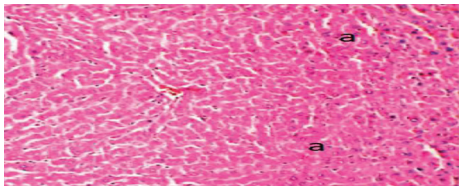
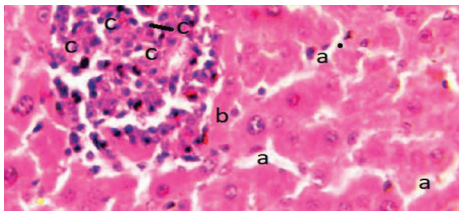
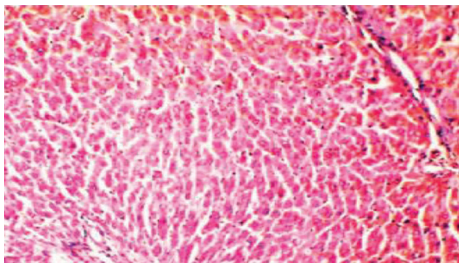
Data expressed as Mean±SEM for six observations $P<0.001$ compared to respective PCM induced group. The data were analyzed by one way ANOVA followed by students newman keuls: [a]$P<0.001$, [b]$P<0.01$. HMERH: Hydromethanolic extract *of R. humilis* fruits

Table 8: Histopathological report of liver

Treatment	Photomicrograph	Report
Normal control received vehicle		Normal control rats showing normal Histomorphological observation
Disease control CCl_4 (1.5 mL/kg)		a. Severe fatty change, b. infiltrates, c. mononuclear, d. multifocal hepatocytes
CCl_4 (1.5 mL/kg)+Silymarin (100 mg/kg)		a. Mild form of vacuolation Within normal limits
CCl_4 (1.5 mL/kg)+HMERH (500 mg/kg)		a. Mild form of vacuolation Within normal limits. b. congestion of the central vein and hepatic sinusoids
='[CCl_4 (1.5 mL/kg)+HMERH 10 mg/kg)		Normal Histomorphological observation
Normal control received vehicle		Normal histopathological structure

(Contd...)

Table 8: *(Continued)*

Treatment	Photomicrograph	Report
Disease control PCM (2 g/kg)		a. Infiltrates, mild to moderate fatty change. b. mononuclear, c. multifocal in the hepatocytes
PCM (2 g/kg)+Silymarin (100 mg/kg)		a. Mild congestion of the central vein and hepatic sinusoids
PCM (2 g/kg)+HMERH (500 mg/kg)		Vaculation, congestion of the central vein, multifocal hepatocytes
PCM (2 g/kg)+HMERH (1000 mg/kg)		Histomorphological observations are within normal limits

metabolic function. Chronic consumption of ethanol, drugs, and chemicals causes liver injury characterized by hepatocytic degeneration and death.[10] Since ancient times, medicinal plants, aromatic plants, and herbs derived natural products or constituents have been used for the treatment of various diseases. In recent years, researchers are mainly focused on fruits and vegetables as a source of medicine due to its abundant nature of phytoconstituents such as alkaloids, polyphenols, flavonoids, phenolic compounds, protein, and pigments (betalains, chlorophylls, curcumin, carotenoids, and anthocyanins).[11]

The ALT, AST, ALP, and TB levels were taken as important serum marker for liver damage.[12] Severe liver lesion was induced by both CCl_4 and PCM. Bilirubin is the degraded product of hemoglobin which is excreted into bile. In liver injury, a lesser quantity will be excreted via bile, resulting in hyper bilirubinemia. The increased level of TB in disease control group is due to the damaged liver, failure to conjugate the bilirubin with glucuronate to become a conjugated bilirubin in the liver.[13] The fruits of *R. humilis* juice contain betalain pigments. The present study was planned to investigate the effect of HMERH fruits as a hepatoprotective agent. Silymarin obtained from the plant *Silybum marianum* (*Asteraceae*) used as the standard drug. Its hepatoprotective nature was due to antioxidant activity.

The tissue level toxicity caused by the CCl_4 and PCM was significantly abolished with HMERH fruits treatment, suggesting that it has a protective effect against liver injury in rats. Trichloromethyl is the free radical formed from the metabolites of CCl_4, which covalently binds to cell proteins followed by a chain reaction of membrane lipid oxidation which leads to cell death. Lipid peroxidation and the following events are the primary causes of CCl_4-induced liver damage. It can be observed by increased level of MDA as well as altered enzymatic role and quantification of endogenous tissue antioxidant enzymes such as SOD, CAT, P, and GSH. In general, our body in build with an effective defense mechanism to overcome or prevent oxidative stress with the help of the above mentioned endogenous antioxidant enzymes.[14] These enzymes support the defense team against the free radicals where SOD, CAT, and GSH are called first line enzymes in the fight against free radicals. These enzymes especially dismutate superoxide radicals an neutralize the reactant hydrogen peroxides and hydroperoxides into innocuous particles. They also additionally incorporate metal particle restricting proteins such as transferrin and ceruloplasmin, which chelate iron and copper respectively, and keeping them from free radical formation.[15] The HMERH fruits reestablished the activities of antioxidant enzymes and attenuate oxidative stress, at least partly or completely by renewing the activities of antioxidant enzymes or by increasing GSH content in the liver. GSH

is a tissue specific detoxifying enzyme which eliminates toxic free radicals by conjugation reaction and prevents cell damage from toxic metabolites.[16] The present study showed significant depletion in hepatic GSH content with only CCl_4 and PCM treated rats, whereas pre-treatement with HMERH prevented the depletion of hepatic GSH level, suggestive of strong antioxidant activity.

The results showed a serum lipids level correlation with the severity of liver damage. An altered lipid profile was found with liver cirrhosis which indicates that reduced biosynthesis of lipid components in the liver. A study report says that condition of chronic hepatitis B and C as well as liver cirrhosis cause impaired lipid metabolism, which leads to reduced level of TC and HDL in patients.[2] Pre-treatment with HMERH extract restored the lipid profile to normal level.

CONCLUSION

The antioxidant properties of HMERH fruit extract may be due to the phytoconstituent betalain. It has strong antioxidant properties and can be used for prevent free radical injury. Other phytoconstients present in *R. humilis* might show more activities which may be revealed in the future studies.

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CONFLICTS OF INTEREST

The authors declare that the contents in this article have no conflicts of interest.

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