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Protective effect of *Mollugo nudicaulis* Lam. on acute liver injury induced by perchloroethylene in experimental rats

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ABSTRACT

Objective: To evaluate the protective effect of ethanol extract of *Mollugo nudicaulis (M. nudicaulis)* against perchloroethylene-induced hepatotoxicity. **Methods:** The hepatoprotective activity of the ethanol extract of *M. nudicaulis* (200 mg/kg body wt) was studied in percholoroethylene (1 000 mg/kg body wt) induced hepatotoxicity in Wistar albino rats. The serum levels of AST, ALT, ALP, bilirubin and the liver content of SOD, CAT, GPx, GST, GSH, vitamin C were assessed to evaluate the hepatoprotective and antioxidant activities of the extract. The activity of the extract was compared with silymarin, a standard reference drug. In addition, serum urea, uric acid and creatinine levels were measured to evaluate the kidney function. The histopathological examination of the liver tissues was observed to support the biochemical parameters. **Results:** The results revealed that the extract significantly ($P<0.05$) restored the serum levels of AST, ALT, ALP, bilirubin and significantly ($P<0.05$) increased the antioxidant enzymes SOD, CAT, GPx, GST, GSH, vitamin C in perchloroethylene-induced rats to its normalcy. The biochemical observations were supported by the histopathological studies of the liver tissues. **Conclusions:** The results led to the conclusion that *M. nudicaulis* possess hepatoprotective and antioxidant activites against perchloroethylene-induced hepatotoxicity in rats.

1. Introduction

Liver, an important organ actively involved in metabolic functions is a frequent target of number of toxicants[1]. In absence of a reliable liver protective drug in the modern medicine, there are number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders[2]. To date, plants continue to be a major source of commercially consumed drugs. Even most synthetic drugs have their origin from natural plant products[3]. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there is not much drug available for the treatment of liver disorders[4]. Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model[5].

Perchloroethylene (PERC), also known as tetrachloroethylene or tetrachloroethene is widely used as a dry cleaning solvent and believed to be a human carcinogen[6]. Metabolism of PERC occurs by cytochrome p450 - dependent oxidative and glutathione (GSH) conjugation. The cytochrome p450 pathway generates tri and dichloroacetate as metabolites of PERC, and these are associated with hepatic toxicity and carcinogenicity[7]. There is an evidence of hepatotoxic caused by PERC, presumably due to reactive metabolic intermediates, lipid peroxidation as a potential mechanism of toxicity[8]. The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against hepatic damage.

Mollugo nudicaulis Lam (*M. nudicaulis*) (Molluginaceae) used in Indian phytotherapy for the treatment of inflammation, urinary disorders and kidney disorders and exhibit higher antioxidant activity[9]. It is also used to treat wounds, cold, cough, fever, and body pain[10]. Our previous study suggests that the ethanol extract of *M. nudicaulis* possess high free radical scavenging activity

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and phytochemical constituents [11]. The present first-time investigation was carried out to study the hepatoprotective activty of the ethanol extract of *M. nudicaulis* against PERC-induced hepatotoxicity. Throughout the study, the ethanol extract of *M. nudicaulis* was compared with silymarin, a standard reference drug.

2. Materials and methods

2.1. Collection and extraction of plant material

Fresh plant materials were collected from the rural areas of Coimbatore, Tamilnadu, India. The plant was authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Tamilnadu Agricultural University Campus, Coimbatore with the voucher number BSI/SRC/5/23/10-11/Tech 420. The plant materials were washed under running water, cut into pieces, air dried and pulverized into fine powder in a grinding machine. 100 g of the powder was then extracted with 500 mL of ethanol, filtered, squeezed off and evaporated under reduced pressure in a rotary evaporator to obtain crude extract.

2.2. Experimental animals

Female Wistar albino rats (120-150 g) procured from Animal house of Karpagam University, Coimbatore were used for the study. The animals were housed in large spacious cage and standard temperature (25 ± 1) ℃ in the animal house. They were fed with standard commercial rat pellets, purified drinking water ad libitum throughout the experimental period. This study was approved by the Institutional Animal Ethics Committee constituted for the purpose of CPCSEA, Govt. of India.

2.3. Experimental methods

The rats were randomized into 5 groups comprising of 6 animals each. The duration of the experimental period was 14 days.

Group I: Control animals; Group II: Control animals treated with PERC (1 000 mg/kg body wt); Group III: Control animals treated with PERC + ethanol extract (200 mg/kg body wt); Group IV: Control animals treated with PERC + silymarin (25 mg/kg body wt); Group V: Control animals treated with ethanol extract of *M. nudicaulis* alone.

PERC (E. Merk, Mumbai, India) was administered orally at a single dose of 1 000mg/kg body wt. This dosage is known to cause hepatotoxins in rats. After 14 days of treatment, the animals were lightly anesthetized with chloroform and sacrificed. Blood samples were collected and the serum was separated by centrifugation for various biochemical analysis namely, AST, ALT, ALP, bilirubin, urea, uric acid and creatinine. The liver was excised immediately and homogenized in ice cold saline (0.9 %) and formalin (10 %) for antioxidant and histopathological studies respectively.

2.4. Biochemical estimations

AST, ALT and ALP[12], bilirubin[13], urea[14], uric acid[15], creatinine[16] were assessed in serum.

2.4.1. Estimation of lipid peroxidation

Lipid peroxidation[17] was estimated and calculated on the basis of the molar extinction coefficient of malondialdehyde (MDA) and expressed in terms of nanomolar of MDA/mg protein.

2.4.2. Antioxidant assays

The enzymatic antioxidants namely superoxide dismutase (SOD)[18], catalase (CAT)[19], glutathione peroxidase (GPx)[20], glutathione-S- transferase (GST)[21] and non enzymatic antioxidants like reduced glutathione (GSH)[22] and vitamin C[23] were evaluated in liver tissue homogenate.

2.5. Statistical analysis

Results were expressed as mean ± SD. The statistical comparison among the groups were performed with one way ANOVA test using a statistical package program (SPSS 10.0) at $P<0.05$ significant level.

3. Results

3.1. Effect of M. nudicaulis on serum biochemical estimations

The results of PERC-induced hepatotoxicity are represented in Table 1. PERC-induced liver damage (Group II) significantly ($P<0.05$) elevated the levels of serum AST, ALT ALP, bilirubin as well as urea, creatinine

Table 1
Effect of ethanolic extract of *M. nudicaulis* on serum biochemical parameters in control and experimental animals.

Groups	AST	ALT	ALP	Bilirubin	Urea	Uric acid	Creatinine
Normal control	18.14 ± 1.91^{a}	28.34 ± 3.19^{a}	150.65 ± 20.42^{a}	0.87 ± 0.08^{a}	26.25 ± 4.03^{a}	9.04 ± 0.59^{d}	1.21 ± 0.36^{a}
PERC control	27.82 ± 1.98^{c}	45.17 ± 3.40^{d}	252.94 ± 23.18^{b}	2.82 ± 0.05^{d}	77.92 ± 17.96^{c}	4.40 ± 0.45^{a}	3.53 ± 0.74^{d}
PERC + PE (200 mg/kg)	19.78 ± 0.80^{ab}	34.77 ± 0.74^{c}	171.14 ± 19.06^{a}	0.98 ± 0.08^{c}	38.33 ± 5.04^{b}	7.20 ± 0.11^{b}	1.61 ± 0.41^{c}
PERC + Silymarin (25 mg/kg)	20.19 ± 0.83^{b}	35.06 ± 1.77^{bc}	169.25 ± 3.81^{a}	0.89 ± 0.03^{b}	31.00 ± 3.35^{ab}	7.80 ± 0.22^{c}	1.25 ± 0.26^{b}
PE alone (200 mg/kg)	18.48 ± 0.64^{a}	30.53 ± 1.16^{ab}	158.09 ± 14.19^{a}	0.86 ± 0.04^{a}	27.12 ± 2.24^{a}	8.68 ± 0.27^{d}	1.22 ± 0.155^{a}

Values are expressed as mean ± SD for six animals, values not sharing common superscript letters (a-e) differ significaatly at $P<0.05$. Units- AST, ALT - μ moles of pyruvate liberated/l; ALP - μ moles of phenol liberated.

and significantly decreased the levels of uric acid. Treatment with the ethanol extractand silymarin (Group IV) significantly (P<0.05) decreased the levels of AST, ALT, ALP, bilirubin as well as urea and creatinine and increased the levels of the uric acid.

3.2. Effect of *M. nudicaulis* on lipid peroxidation in liver tissues

The effect of *M. nudicaulis* on lipid peroxidation in liver tissues was shown in Table 2. The MDA content was significantly (P<0.05) increased in PERC-induced control animals (Group II). Treatment with the ethanol extract of *M. nudicaulis* significantly (P<0.05) and silymarin (Group IV) decreased the levels of MDA content (Group III).

3.3. Effect of *M. nudicaulis* on enzymatic antioxidant activity in liver tissues

3.3.1. Effect of the ethanol extract of *M. nudicaulis* on SOD activity

The activities of SOD in the liver homogenates of all the groups are shown in Table 2. The SOD activity in PERC-induced control animals (Group II) was reduced compared to control animals (Group I). Treatment with the ethanol extract of *M. nudicaulis* (Group III) enhanced the SOD value, compared to PERC-induced control animals (Group II). Silymarin treated PERC-induced animals (Group IV) also showed an augmented SOD value compared to its corresponding PERC-induced control animals (Group II).

3.3.2. Effect of the ethanol extract of *M. nudicaulis* on CAT activity

The CAT activity levels in all the group of animals are shown in Table 2. The CAT activity in liver homogenates of PERC-induced control animals (Group II) was significantly (P<0.05) lower than that of group I. Treatment of the animals with the ethanol extract (Group III) and silymarin (Group IV) significantly (P<0.05) increased the CAT activity compared to the PERC-induced control animals (Group II). CAT level in the ethanol extract alone treated animals (Group V) was almost close to control animals (Group I).

3.3.3. Effect of the ethanol extract of *M. nudicaulis* on GPx activity

Table 2 shows the GPx activity in the liver homogenate of all the groups of animals. A significant reduction in GPx activity was observed in PERC-induced animals (Group II) when compared to that of control animals (Group I). Treatment with the ethanol extract (Group III) and silymarin (Group IV) significantly increased the GPx activity. GPx level in the ethanol extract alone treated animals (Group V) was almost close to control animals (Group I).

3.3.4. Effect of the ethanol extract of *M. nudicaulis* on GST activity

Table 2 shows the GST activity in the liver homogenate of all the group of animals. The GST activity in liver homogenates of PERC-induced control animals (Group II) was significantly (P<0.05) reduced than that of control animals (Group I). Treatment with the ethanol extract (Group III) and silymarin (Group IV) significantly (P<0.05) increased the GST activity compared to the PERC-induced control animals (Group II). GST level in the ethanol extract alone treated animals (Group V) was almost close to control animals (Group I).

3.4. Effect of *M. nudicaulis* on non-enzymatic antioxidant activity in liver tissues

The activities of GSH and vitamin C in the liver homogenates of all the groups are shown in Table 2. There was a significant reduction in the activities of GSH and vitamin C in PERC-induced control animals (Group II). Treatment with the ethanol extract (Group III) and silymarin (Group IV) significantly (P<0.05) increased the GSH and vitamin C activity compared to the PERC-induced control animals (Group II). There was no significant difference between the ethanol extract alone treated animals (Group V) and control animals (Group I).

3.5. Histopathological studies

Figure 1 shows the histopathological examination of the liver sections of the experimental animals. The control group (Group I) shows no obvious abnormality (Figure 1a).

Table 2

Effect of ethanolic extract of *M. nudicaulis* on the activities of enzymatic/non-enzymatic antioxidants in liver of control and experimental animals.

Groups	Enzymatic antioxidants					Non-enzymatic antioxidants	
	LPO	SOD	CAT	GPx	GST	GSH	Vit C
Normal control	2.54 ± 0.10^a	6.45 ± 0.08^d	1.81 ± 0.04^d	1.82 ± 0.05^e	102.48 ± 3.02^d	12.47 ± 1.03^c	1.69 ± 0.05^d
PERC control	5.31 ± 0.11^b	3.11 ± 0.10^a	0.82 ± 0.05^a	0.76 ± 0.06^a	46.67 ± 2.27^a	8.39 ± 0.19^a	0.95 ± 0.03^a
PERC + PE (200 mg/kg)	3.15 ± 0.32^c	5.22 ± 0.11^b	1.33 ± 0.04^b	1.23 ± 0.03^b	84.34 ± 5.17^b	11.07 ± 0.5^b	1.46 ± 0.08^b
PERC + Silymarin (25mg/kg)	2.92 ± 0.13^d	6.04 ± 0.07^c	1.64 ± 0.05^c	1.41 ± 0.02^c	86.86 ± 4.99^c	11.58 ± 0.41^b	1.61 ± 0.06^c
PE alone (200mg/kg)	2.53 ± 0.08^a	6.35 ± 0.06^d	1.82 ± 0.03^d	1.72 ± 0.08^d	101.04 ± 3.10^d	12.40 ± 0.58^c	1.68 ± 0.05^d

Values are expressed as mean ± SD for six animals, values not sharing common superscript letters (a-e) differ significantly at P<0.05. LPO - nM/mg protein; SOD - inhibition of 50% nitrite formation/min/mg protein; CAT - μ mol of H_2O_2 consumed/min/mg protein; GPx - μ mol of glutathione oxidized/min/mg protein; GST- μ mole of glutathione utilized/min/ mg protein.

The liver section of PERC–induced group (Group II) shows minimal periportal lymphocytic infiltrate with extension of inflammation and necrosis of hepatocytes in focal areas (Figure 1 b). Treatment with the ethanol extract (Group III) shows very minimal diffuse fatty change and majority of liver appears normal (Figure 1 c). In silymarin treated group (Group IV) very minimal non specific periportal lymphocytoic infiltrate and minimal diffuse fatty change was found (Figure 1d). *M. nudicaulis* alone treated group (Group V) shows no obvious abnormality (Figure 1 e).

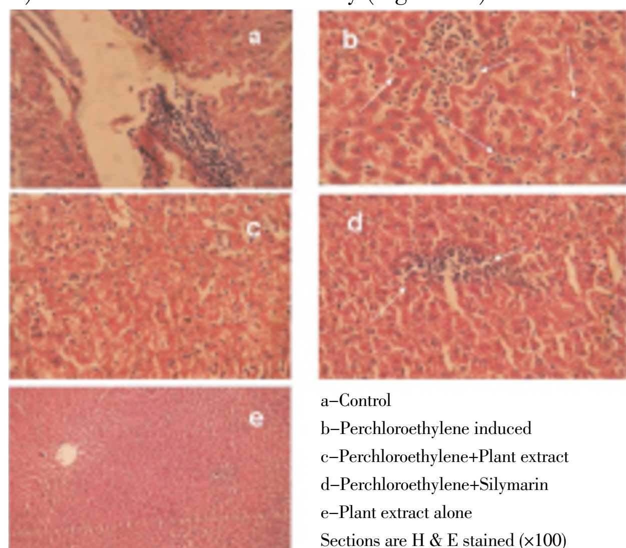

Figure 1. Histopathology of Liver.
a – Control group shows no obvious abnormality
b – Perchloroethylene treated rats shows minimal periportal lymphocytic infiltrate with extension of inflammation and necrosis of hepatocytes in focal areas.
c – Group III intoxicated rats treated with *M. nudicaulis* shows very minimal diffuse fatty change and majority of liver appears normal
d – Group IV silymarin treated group shows very minimal non specific periportal lymphocytoic infiltrate and minimal diffuse fatty change
e – Group V *M. nudicaulis* alone treated group shows no obvious abnormality

4. Discussion

The hepatotoxic, nephrotoxic, and carcinogenic effects of PERC depend on its metabolism to reactive metabolites. Cytochrome p450 dependent oxidation and GSH conjugation are two principal pathways of PERC metabolism that occur in liver and kidney leading to the generation of reactive metabolites which may covalently bind to cellular macromolecules. PERC oxidation is catalyzed primarily by CYP2E1 to form PERC–epoxide and further to trichloroacetyl chloride, which can react with amino groups in macromolecules resulting in hepatotoxicity or with water to give trichloroacetic acid[24].

Serum AST, ALT and ALP are the most sensitive markers employed in the diagnosis of hepatic damage because they are cytoplasmic enzymes released into circulation after cellular damage[25]. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage[26]. Administration of *M. nudicaulis* reduced the level of serum liver markers towards the normal level is an indication of stabilization of plasma membrane and protection of hepatic tissue damage. The marked elevation of serum bilirubin level in serum of PERC–induced animals was significantly decreased in the ethanol extract treated animals. Bilirubin is the conventional indicator of liver diseases[27]. Bilirubin an endogenous organic anion binds reversibly to albumin and it is transported to the liver, conjugates with the glucouronic acid and excreted in the bile. Hepatobiliary disease is indicated when total bilirubin exceeds the upper limit of normal[28]. The bilirubin lowering ability of the ethanol extract in PERC–induced animals further indicate the hepatoprotective nature of *M. nudicaulis* on hepatocytes when compared to the PERC–induced animals.

Lipid peroxidation plays an important role in carcinogenesis and may lead to the formation of several toxic products, such as malondialdehyde (MDE) and 4– hydroxynonenal. These products attack cellular targets including DNA, thereby inducing mutagenecity and carcinogenicity[29]. The rats treated with single dose of PERC developed a significant hepatic damage and oxidative stress, which was observed from a substantial increase in the lipid peroxidation[30]. In the present study, the observed increase in lipid peroxidation and decrease in the levels of enzymatic antioxidants (SOD, CAT, GPx and GST) and non–enzymatic antioxidants (GSH and vitamin C) during PERC administration indicate the complete disruption of the antioxidant defense mechanism of the liver. SOD, CAT and GPx play an important role in the protection of cell membranes against oxidative damage[31]. SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues. CAT is a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical–induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide[32].

The GSH antioxidant system consists of an array of non–enzymic and enzymic reaction pathways involving the neutralization of free radical species. Preturbation of the GSH status of a biological system has been reported to lead to serious consequence[33]. GPx utilizes it for the decomposition of lipid hydroperoxides and other reactive oxygen species (ROS) and GST maximizes the conjugation of free radicals and various lipid hydroperoxides to GSH to form water–

soluble products that can be easily excreted out[34]. The administration of *M. nudicaulis* display a reduction in lipid peroxidation and elevation of tissue antioxidant defense enzyme activity levels indicating that the *M. nudicaulis* could reduce the free radical generation and it may be due to the potent antioxidant activity of ethanol extract of *M. nudicaulis*. Thus the reduced activity of enzymatic and non-enzymatic antioxidants could be due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes.

If there is an increase or decrease in the level of renal marker enzymes, it indicates the dysfunction of kidney[35] that is accompanied by an increased urea and subsequently creatinine level[36]. Uric acid, the metabolic end product of purine metabolism, has proven to be a selective antioxidant, capable especially of reacting with free radicals and hypochlorous acid[37]. The reduced level of uric acid in hepatotoxicity conditions may be due to the increased utilization of uric acid against increased production of the free radicals[32]. The reversal of altered uric acid level to near normal could be due to strong antioxidant property of *M. nudicaulis*, which contributes to its antioxidant potency. The administration of *M. nudicaulis* showed its ability to restore the normal functional status of the kidney. The results were found comparable to silymarin.

The hepatoprotective potential of several herbal medicines has been clinically evaluated[38]. Numerous studies also revealed that the herbal medicines can alter the toxic condition of the hepatocytes induced by chemicals[39-41]. In our previous studies that were investigated in our laboratory also showed that medicinal plants protect the liver against hepatotoxicity induced by various chemicals[42-44].

The results of this study demonstrated that *M. nudicaulis* has significant action on PERC-induced hepatotoxicity. The hepatoprotective effect of *M. nudicaulis* may be due to its phytochemical constituents and antioxidant properties. Further study on the plant can be extended for the isolation of active constituents, structural determination and mode of action of the extract.

Conflict of interest statement

We declare that we have no conflict of interest.

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