HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *HEDYOTIS CORYMBOSA* ON PERCHLOROETHYLENE INDUCED RATS

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Summary

The present study was designed to evaluate the hepatoprotective effect of *Hedyotis corymbosa* belongs (family - Rubiaceae) on perchloroethylene induced damage in rat liver. The liver marker enzymes were estimated in serum samples and the lipid peroxidation levels and antioxidant status were analyzed in liver homogenate. Perchloroethylene induction (1000mg/kg b.wt) significantly increased the liver marker enzymes, lipid peroxidation levels and significantly decreased the antioxidant status. Treatment of ethanolic extract of *Hedyotis corymbosa* at dose of (400 mg/kg b.wt) was administered orally once daily for ten days decreased the liver marker enzyme levels (AST, ALT and LDH) and lipid peroxidation with increase in antioxidant enzyme levels. The results of this study strongly indicate that *Hedyotis corymbosa* have potent hepatoprotective action against perchloroethylene induced hepatic damage in rats.

Keywords: *Hedyotis corymbosa*, Lipid peroxidation, Liver marker enzymes, and perchloroethylene

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Introduction

Perchloroethylene (tetrachloroethylene, Perc) is a solvent used in dry cleaning operations and industrial applications such as metal degreasing. It is classified as a group 2A carcinogen (probably carcinogenic to humans) by IARC [1]. Perc has been found to produce increases in hepatocellular carcinomas and/or adenomas in mice in chronic inhalation bioassays [2, 3] and is classified as "reasonably anticipated to be a human carcinogen" by the National Toxicology Program [4]and "probably carcinogenic to humans" by the International Agency for Research on Cancer [1]. Perc is metabolized primarily to trichloroacetic acid (TCA), which is also a mouse hepatocarcinogen [5, 6, 7, 8]. Higher incidence of nephropathy [9] and urinary tract cancer [10] were observed in dry cleaning workers exposed to Perc. Animal studies showed that Perc is hepatocarcinogenic in both genders of B6C3F1 mice [11] and a kidney carcinogen in rats [12].

The hepatotoxic, nephrotoxic, and carcinogenic effects of Perc depend on its metabolism to reactive metabolites [13, 14]. Cytochrome p450 dependent oxidation and glutathione (GSH) conjugation are two principal pathways of Perc metabolism that occur in liver and kidney of mice [15] leading to the generation of reactive metabolites which may covalently bind to cellular macromolecules [16]. 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 (TCA) [17].

Hedyotis corymbosa (L.) Lam. Syn. *Oldenlandia corymbosa*(L.) Lam. (Rubiaceae) is a weedy herb, widely distributed throughout India. It is commonly known as 'Parppatakapullu' in traditional medicine of Kerala. *Hedyotis corymbosa* is extensively used in modern Chinese practice for the treatment of viral infections, cancer, syndromes involving "toxic heat", acne, boils, skin ailments, appendicitis, hepatitis, eye diseases and bleeding [18]. The plant is used for treating venomous bites. It is bitter, acrid, cooling, febrifugal, pectoral, anthelmintic, diuretic, depurative, diaphoretic, expectorant, digestive and has stomachic properties [19]. It is given in jaundice, and other diseases of the liver, heat eruptions, vitiated conditions of pitta, hyperdypsia, giddiness, dyspepsia, flatulence,

colic, constipation, helminthiasis, leprosy, skin diseases, cough, bronchitis, necrosis, nervous depression caused by deranged bile and hepatopathy [20]. Recently there have been many studies on traditional medicines, attempting to develop new drugs for hepatitis from them. The hepatoprotective effects of *Hedyotis corymbosa* on carbon tetrachloride (CCl_4)-induced liver damage in rats have been investigated [21,22]. The hepatoprotective effect of the methanolic extract of *Hedyotis corymbosa* on paracetamol overdose-induced liver damage in Wistar rats also have been investigated [23]. The objective of the present investigation was to determine the hepatoprotective effect of ethanolic extract of *Hedyotis corymbosa* on perchloroethylene induced hepatotoxicity in rats.

Materials and Methods

Animals

Wistar strains of female albino rats, weighing 140-160g, were procured from Karpagam University, Animal house, Coimbatore, India. They were housed in standard rodent diet (M/S Hindustan Lever Ltd., Mumbai, India) and water *ad libitum* (Aquaguard filter water). The animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India.

Drug and Chemicals

The whole plant of *Hedyotis corymbosa* (Rubiaceae) was collected from in and around Coimbatore, The plant was authenticated by Dr.R.Gopalan, Scientist, Botanical survey of India, Southern Circle, Coimbatore. The voucher specimen was deposited at the herbarium of Karpagam University, Coimbatore. The whole plants were dried crushed and finally yielding a dry residue. The average percentage yield of HCE was found to be 0.42% w/v. The ethanolic extract of *Hedyotis corymbosa* was used as an oral dose of 400mg/kg b.wt, orally. Perchloroethylene and thiobarbutric acid were obtained from Sigma- Aldrich. St.Louis, USA. All other reagents used were purchased locally.

Experimental protocol

The animals were randomly allocated into four groups (six rats in each group). The first group, the control group. The second group perchloroethylene group, was treated with a single dose of 1000mg/kg b.wt i.p).The third group, perchloroethylene + ethanolic extract of *Hedyotis corymbosa* (400mg/kg b.wt) orally was started a day after the injection for a period of 10 days. In the fourth group the Ethanolic extract of *Hedyotis corymbosa* alone was injected orally for 10 days. The animals were anesthetized using chloroform and sacrificed. Blood was collected directly from the heart of each animal and the clot was centrifuged for 15-20 minutes at 2000 rpm to separate serum and the liver was washed with saline, both were used for biochemical analysis.

Biochemical Analysis

The liver marker enzymes like Alanine aminotransferase, Aspartate amino transferase and lactate dehydrogenase were assayed by King Method [24]. In the hepatic tissue samples lipid peroxidation effect was studied by the modified methods [25]. Malondialdehyde (MDA), formed as an end product of the peroxidation of lipids served as an index of oxidative stress. The antioxidant enzymes Superoxide dismutase was assayed according to the method of Misra and Fridovich [26]. The unit of enzyme activity is defined as the enzyme required to give 50% inhibition of epinephrine to adenochrome. Catalase was assayed by the method of Sinha [27].In this method, dichromate in acetic acid was reduced to chromic acetate. This was measured colorimetrically at 610nm. Glutathione peroxidase was assayed by the method of Rotruck et.al., (28) here the reaction between glutathione remaining after the action of GPx and 5,5'-dithiobis-(2-nitrobenzoic acid) to form a complex that absorbs at 412 nm.

Statistical Analysis

All biochemical values were illustrated as Mean ± Standard Deviation (S.D) for both control and experimental animals. The statistical significance of values between the four groups was analyzed using student “t” test.

Results

Table 1 shows the activities of alanine transaminase, aspartate transaminase, and lactate dehydrogenase in serum of normal, perchloroethylene control and treated groups. The activities of alanine transaminase, aspartate transaminase and lactate dehydrogenase in serum significantly increased in perchloroethylene control group compared to normal group. The levels of the above enzymes were significantly increased on treatment with *Hedyotis corymbosa.*

Table 1.The Activity of Liver Marker Enzymes in serum of control and experimental groups

Parameter Particulars	Control	Perchloroethylene (1000mg/kg/b.wt.)	Perchloroethylene + *Hedyotis corymbosa* (400mg/kg/b.wt)	*Hedyotis corymbosa* (400mg/kg/bwt)
Alanine Transaminase (U/dl)	87.21±6.40	291.23 ± 5.90[a]	138.72 ± 7.38[b]	89.42±5.60[NS]
Aspartate Transaminase (U/dl)	48.62± 6.10	172.32±5.42[a]	64.31±5.43[b]	46.72±5.60[NS]
Lactate dehydrogenase (IU/L)	41.50±4.76	66.06±3.71[a]	42.33±4.13[b]	42.74±4.38[NS]

Values are the Mean±S.D. *n*=6, p<0.01 NS (Not significant); a-Compared with control group, b-Compared with perchloroethylene induced group, NS- Compared with control group.

Table 2 shows the levels of lipid peroxidation, superoxide dismutase, catalase, and glutathione peroxidase in liver homogenate of control and experimental groups In perchloroethylene induced group the MDA levels significantly increased. Peroxy radicals are important agents that mediate lipid peroxidation there by damaging cell membrane where as the antioxidant enzymes like, superoxide dismutase, catalase and glutathione peroxidase levels were signigicantly decreased. By the administration of ethanolic extract of *Hedyotis corymbosa* to perchloroethylene induced rats the antioxidant enzyme levels were restored to near normal.

Table 2.The Activity of lipid peroxidation levels and Liver antioxidant Enzymes in liver sample of control and experimental groups

Particulars	Control	Perchloroethylene (1000mg/kg/b.wt.)	Perchloroethylene + *Hedyotis corymbosa* (400mg/kg/b.wt)	*Hedyotis corymbosa* (400mg/kg/bwt)
Lipid peroxidation (MDA formed per mg protein)	0.86±0.09	2.17 ±0.18[a]	1.78±0.15[b]	1.0 ±0.12[NS]
Superoxide Dismutase (U/mg protein)	5.76±0.38	1.64±0.21[a]	3.20±0.64[b]	4.89±0.68[NS]
Catalase (U/mg protein)	62.46±0.43	40.67±0.62[a]	56.85±0.86[b]	58.60±0.24[NS]
Glutathione peroxidase (U/mg protein)	31.62± 0.70	22.32±0.42[a]	26.76±0.53[b]	28.72±0.47[NS]

Values are the Mean±S.D, *n*=6. p<0.01 NS (Not significant); a- Compared with control group, b- Compared with perchloroethylene induced group, Ns- Compared with control group.

Discussion

In the present study it was observed that the animals treated with perchloroethylene resulted in the significant hepatic damage as shown by the elevated levels of marker enzymes such as ALT, AST, and LDH. Excessive absorption of PCE can produce depression of the central nervous system and hepatic and renal damage [29]. When liver cell plasma is damaged, a variety of enzymes located normally in cytosol is released into the blood, thereby causing increased enzyme levels in the serum. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. The results are in agreement with the commonly accepted view that serum level of transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes [30].

Metabolism of perchloroethylene (Perc) occurs by cytochrome p450-dependent oxidation 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 [24].

Previous studies have shown that administration of dichloroacetate and tri-chloroacetate increased lipid peroxidation, when administered acutely to naive animals [31]. This also induces a variety of changes in intermediary metabolism, but their effects are quite distinct in the fact that dichloroacetae has major effects on carbohydrate metabolism, whereas tri-chloroacetate and other peroxisome proliferators primarily affect lipid metabolism. [32, 33].

Free radical production in cells is relatively low in normal conditions, given the various and very active defense systems including enzymic and nonenzymic antioxidant enzymes administration of perchloroethylene caused a significant inhibition of glutathione peroxidase [34]. A defective antioxidant defense system in PER administered mice was evidenced by the low level of enzymic antioxidants (SOD, CAT, GPx). SOD is an endogenous enzymatic scavenger which can counterbalance the oxidative destruction of free radicals. Most of the SOD in tissues of cytoplasmic origin and contains Cu and Zn an essential prosthetic groups. The decrease in SOD after perchloroethylene administration may be attributable to an interaction between Cu and Zn with high levels of perchloroethylene in tissues. Potential hepatoprotective agents therefore include either free radical scavenging property or agents which are capable of augmenting the activity of antioxidant enzymes (SOD, CAT). In our present study treatment with ethanolic extract of *Hedyotis corymbosa* the antioxidant enzyme levels significantly increased. The methanolic extract of *H.Corymbosa* has potent antilipid peroxidant effect in paracetamol overdose-induced induced rats [23].The present study revealed that HCE also has potent antilipid peroxidant effect.

Nine iridoid glycoside derivatives were isolated from the aerial parts of *Hedyotis corymbosa* [35].The iridoid glycosides isolated from *Picrorrhiza kurroa* showed marked protective action on liver against CCl_4 intoxicated rats by enhancing the choleretic activity and also reduced the levels of ALT and AST [36]. *H.Corymbosa* has also been reported to contain oleanolic acid, ursolic acid and γ-sitosterol [37]. Ursolic acid exhibited potent hepatoprotective effects [38]. Oleanolic acid has been reported to increase the antioxidant components in the liver, such as glucuronosyl transferase towards acetaminophen in mice. It also increased maintained the hepatic glutathione, which plays an important role in protecting against acetaminophen- induced liver injury [39].

In the present study, it is observed that the bioactive fractions present in *Hedyotis corymbosa* are responsible for the marked hepatoprotective effects, observed in present study.

In conclusion, the result of this study seems to confirm that the ethanolic extract of *H. Corymbosa* has a potent hepatoprotective action upon perchloroethylene-induced hepatic damage in rats and possess antilipid peroxidative and free radical scavenging activities. The present study thus justifies the traditional use of *Hedyotis corymbosa* in the treatment of liver disease and also point out that *Hedyotis corymbosa* warrants future detailed investigation as a promising hepatoprotective agent.

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