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Research Article

HEPATOPROTECTIVE EFFECT OF *HEDYOTIS LESCHENAULTIANA* DC, ETHANOL EXTRACT IN CCL_4 INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT

Objective: The intention of this study is to explore the hepatoprotective potential of hepatoprotective potential of ethanol extract of *Hedyotis leschenaultiana* whole plant in carbon tetrachloride (CCl_4) induced hepatoprotective rats.

Methods: Hepatotoxicity was induced in male wistar rats by intraperitoneal infection of CCl_4 (2.5 ml/kg body weight for 14 days). The ethanol extract of *H. leschenaultiana* whole plant was administered to the experimental rats (100, 200, and 300 mg/kg body weight for 14 days). Silymarin (100 mg/kg) was given as a reference standard drug. In hepatotoxic rats, liver damage was studied by assessing parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), total, conjugated and unconjugated bilirubin, gamma-glutamyl transferase, concentration of proteins, and antioxidants in serum.

Results: Administration of hepatotoxins (CCl_4) showed significant elevation of SGOT, SGPT, serum ALP, total bilirubin, conjugated, unconjugated, and lipid peroxidation. Treatment with *H. leschenaultiana* (100, 200 and 300 mg/kg) significantly reduced the above-mentioned parameters. Regarding antioxidant activity, the ethanol extract of *H. leschenaultiana* exhibited a significant effect showing increasing levels of superoxide dismutase, catalase, glutathione peroxidase, reduced and glutathione, and glutathione reductase by reducing malondialdehyde levels.

Conclusion: The ethanol extract of *H. leschenaultiana* have a significant effect on the CCl_4 induced hepatotoxic animal models. Moreover, it is suggested that *H. leschenaultiana* can be used as a safe, cheap and effective alternative chemopreventive and protective against in the management of liver diseases.

Keywords: *H. leschenaultiana*, Bilirubins, Hepatotoxicity, Gamma-glutamyl transferase, Carbon tetrachloride, Melondialdehyde.

INTRODUCTION

The liver is one of the major organs in the body responsible for maintaining the homeostasis of body. It is having a significant role in growth, fight against disease, nutrient supply, energy provision, reproduction and it gives protection against the hazards of harmful drugs and chemicals. Because of the complex nature, it is susceptible to many adverse effects from a wide variety of things such as alcohol, infections from hepatitis viruses, cancer, and other metabolic disorders [1]. The liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment of its functions may lead to many implications on one's health. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects, whereas herbs play a role in the management of various liver diseases. Many fold remedies from plant origin have been long used for the treatment of liver diseases. This is one of the reasons for many people in worldwide including those in developed countries turning complementary and alternative medicine. Many traditional remedies employ herbal drugs for the treatment of liver ailments [2]. Herbs play a major role in the management of various liver disorders along with other system associated diseases. Plant-derived natural products such as flavonoids, terpenoids, and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity [2,3]. Hence, people are looking at the traditional systems of medicine for remedies to treat hepatic disorders.

However, no scientific investigation is available regarding the hepatoprotective effect of *Hedyotis leschenaultiana* whole plant. Since antioxidants are known to reduce the development of chemically induced liver damage, the effect of ethanol extract of the whole plant of *H. leschenaultiana* has been evaluated for hepatoprotective activity against carbon tetrachloride (CCl_4) induced liver damage using rat as an experimental animal.

METHODS

Collection of plant sample

The whole plant of *H. leschenaultiana* DC was collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu, India. The plant was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu, India.

Preparation of plant extract for phytochemical screening and hepatoprotective studies

The whole plant of *H. leschenaultiana* was shade dried at room temperature, and the dried leaves were powdered in a Wiley mill. 100 g of powdered *H. leschenaultiana* whole plant was packed in a soxhlet apparatus and extracted with ethanol. The extracts were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures [4-6]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extracts were used for hepatoprotective studies.

Animals

Normal healthy male wistar albino rats (180-240 g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25°C±2°C) and

light and dark (12:12 hrs). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan Lever Ltd., Mumbai, Maharashtra, India) and water *ad-libitum.* The experimental protocol was approved by the Institutional Animal Ethical Committee of Kongunadu Arts and Science College (Reg. No: 659/02/a CPCESEA) Coimbatore, India.

Acute toxicity studies

An acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [7]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5 mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 up to 2000 mg/kg body weight.

Experimental design

In this investigation, a total of 30 rats (25 CCl_4 hepatic toxicity induced rats and 5 normal rats) were taken and divided into six groups of 5 rats each. Group I: Rats received normal saline was served as a normal control. Group II: CCl_4 hepatic toxicity induced control: Rats received 2.5 ml/kg body weight of CCl_4 for 14 days. Group III: Liver injured rats received ethanol extract of the whole plant of *H. leschenaultiana* at the dose of 100 mg/kg body weight for 14 days. Group IV: Liver injured rats received ethanol extract of whole plant of *H. leschenaultiana* at the dose of 200 mg/kg body weight for 14 days. Group V: Liver injured rats received ethanol extract of the whole plant of *H. leschenaultiana* at the dose of 300 mg/kg body weight for 14 days. Group VI: Liver injured rats received standard drug silymarin at the dose of 100 mg/kg body weight for 14 days.

Biochemical analysis

The animals were sacrificed at the end of the experimental period of 7-days by decapitation. Blood was collected, sera separated by centrifugation at 3000 g for 10 minutes. Serum protein [8] and serum albumins were determined quantitatively by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were measured spectrophotometrically using the method of Reitman and Frankel [9]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [10]. Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw [11]. The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated bilirubin concentrations. Gamma-glutamyl transferase (GGT) was estimated by the method of Szasz [12]. Quantitative estimation of melondialdehyde (MDA) formation was done by determining the concentration of thiobarbituric acid reactive substances in plasma by the method of Satoh [13]. Enzymatic antioxidants, superoxide dismutase (SOD) [14], catalase (CAT) [15], glutathione reductase (GRD) [16], reduced and glutathione (GSH) [17], and glutathione peroxidase (GPx) [18] were also assayed in erythrocytes.

Statistical analysis

The data were expressed as the mean±standard error of mean. The difference among the means has been analyzed by one-way ANOVA. $p<0.01$ and $p<0.05$ were considered as a statistical significance using SPSS Software.

RESULTS

Preliminary phytochemical screening

The phytochemical screening of ethanol extract of the whole plant of *H. leschenaultiana* revealed the presence of alkaloid, catechin, coumarin, flavonoid, tannin, saponin, steroid, phenol, glycoside, terpenoid, and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of whole plant of *H. leschenaultiana*.

Table 1: Effect of *H. leschenaultiana* whole plant extract on the body weight of in the normal, liver damaged and drug treated rats

Treatment	Initial body weight (g)	Final body weight (g)	Mean weight Gain (G↑)/ loss (L↓) (g)	% Difference
Group I	216.32±6.45	224.93±5.86	8.61↑	3.98
Group II	206.54±5.94	184.39±6.21	22.15↓**	10.72
Group III	198.51±6.23	206.11±5.94	7.60↑$^{ns, a}$	3.8
Group IV	209.33±5.14	214.66±6.13	5.33↑$^{ns, aa}$	2.6
Group V	191.56±4.88	199.26±5.64	7.70↑$^{ns, a}$	4.0
Group VI	201.93±7.22	211.45±6.29	9.52↑$^{ns, a}$	4.7

Values are mean±SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. ** $p<0.01$ as compared with normal control to liver damaged control: $^{a}p<0.05$; $^{aa}p<0.01$ as compared with liver damaged control to drug treated animal. ns: Not significant. SD: Standard deviation, *H. leschenaultiana: Hedyotis leschenaultiana*

Body weight

The effect of ethanol extract of *H. leschenaultiana* on body weight of the normal, CCl_4 intoxicated and drug treated rats are shown in Table 1. The administration of CCl_4 caused a significant ($p<0.05$) decrease in the body weight of rats as compared with the control rats. The animals treated with whole plant extracts of *H. leschenaultiana* (200 and 300 mg/kg) also gained weight during the experimental period.

Biochemical parameters

Table 2 shows the effect of ethanol extract of *H. leschenaultiana* on serum total protein, albumin, globulin, A/G ratio, serum transaminases, and ALPs in CCl_4 intoxicated rats. There was a significant ($p<0.01$) increase in SGOT, SGPT, and serum ALP levels in CCl_4 intoxicated group (Group II) compared to the normal control group (Group I). The total protein and albumin levels were significantly ($p<0.01$) decreased to 6.29 and 3.76 g/dl in CCl_4 intoxicated rats from the levels of 8.65 and 4.85 g/dl, respectively, in the normal group. The ethanol extract of *H. leschenaultiana* at the dose of 300 mg/kg orally significantly decreased the elevated serum marker enzymes and reversed the altered total protein and albumin to almost normal level.

Total, conjugated, unconjugated and GGT levels

The effect of ethanol extract of *H. leschenaultiana* on total, conjugated and unconjugated bilirubin are shown in Table 3. A significant elevation of total, conjugated, unconjugated bilirubin, and GGT in the serum of CCl_4 intoxicated group (Group II) when compared to normal control (Group I) were observed. The ethanol extract of *H. leschenaultiana* at the dose 300 mg/kg reduced the levels of total, conjugated and unconjugated bilirubin (Group V). The decreases in the concentration of total bilirubin, conjugated bilirubin, unconjugated bilirubin and GGT were found to be greater in 300 mg/kg (Group V) followed by Group VI and Group IV and Group V.

Antioxidant activity

The effect of ethanol extract of *H. leschenaultiana* on lipid peroxidation (LPO), GPx, GRD, SOD, CAT, and GSH activity are shown in Table 4. LPO level was significantly ($p<0.01$) increased and GPx, GRD, SOD, and CAT activity were significantly ($p<0.01$) decreased in CCl_4 intoxicated rats when compared with those of the animals in normal control group. Rats treated with ethanol extract of *H. leschenaultiana* at the doses of 300 mg/kg significantly decreased the elevated LPO levels and restored the altered GPx, GRD, SOD, CAT and reduced glutathione levels toward the normal levels in a dose-dependent manner. The results are well comparable with silymarin (standard drug) treated group.

Table 2: Effect of *H. leschenaultiana* whole plant extract on the serum protein, albumin, globulin concentration and SGOT, SGPT and serum ALP enzyme activity in the normal, liver damaged and drug treated rats

Groups	Parameters						
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Group I	8.65±0.14	4.85±0.16	3.80±0.21	1.27:1	18.11±1.96	21.56±0.91	146.33±2.54
Group II	6.29±0.11*	3.76±0.16	2.53±0.16*	1.48:1	116.41±3.95**	143.59±4.54**	227.16±5.13**
Group III	8.18±0.19	4.26±0.61	3.92±0.27	1.08:1	73.54±2.33*[a]	68.29±4.15*[aa]	174.14±2.93*[a]
Group IV	8.29±0.31	4.93±0.29	3.36±0.71	1.46:1	34.16±2.65*[aa]	31.59±3.68[ns]	163.22±3.63*[a]
Group V	7.93±0.26[a]	4.28±0.17	3.65±0.39	1.17:1	24.63±1.84[ns]	20.51±1.08[aa]	154.56±2.81[ns, aa]
Group VI	8.41±0.19[a]	4.84±0.27	3.57±0.43	1.35:1	19.85±0.98[ns]	21.33±1.96[aa]	139.46±1.86[ns, aa]

Values are mean±SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *p<0.05; ** p<0.01 as compared with normal control to liver damaged control: [a]p<0.05; [aa]p<0.01; [aaa]p<0.001 as compared with liver damaged control to drug treated animal. ns: Not significant. SD: Standard deviation, *H. leschenaultiana: Hedyotis leschenaultiana*, ALP: Alkaline phosphatase, SGOT: Serum glutamate oxalo acetate transaminase, SGPT: Serum glutamate pyruvate transaminase

Table 3: Effect of *H. leschenaultiana* whole plant extract on the serum total, conjugated, unconjugated bilirubin and GGTP levels in the normal control, liver injured and drug treated rats

Groups	Parameters			
	Total bilirubin (mg/dl)	Conjugated (mg/dl)	Unconjugated (mg/dl)	GGTP (U/L)
Group I	0.87±0.04	0.25±0.07	0.62±0.05	8.75±0.59
Group II	3.92±0.24**	2.24±0.34**	1.68±0.13*	30.16±0.98**
Group III	1.89±0.03*	1.24±0.04*	0.65±0.03[ns]	21.63±0.35*
Group IV	0.98±0.01[ns, a]	0.39±0.01[a]	0.59±0.06[a]	10.55±0.94[a]
Group V	0.75±0.07[ns, aa]	0.21±0.03[aa]	0.44±0.03[aa]	7.69±0.33[aa]
Group VI	0.89±0.04[a]	0.28±0.07[aa]	0.61±0.08[a]	9.31±0.74[a]

Values are mean±SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *p<0.05; ** p<0.01 as compared with normal control to liver damaged control: [a]p<0.05; [aa]p<0.01 as compared with liver damaged control to drug treated animal. ns: Not significant. SD: Standard deviation, GGPT: Gamma-glutamictranspeptidase, *H. leschenaultiana*: *Hedyotis leschenaultiana*

DISCUSSION

The liver is one of the largest organs in the human body and the chief site for internal metabolism and excretion. Hence, it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, and energy provision [19]. The major functions of the liver are carbohydrate, protein, and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, liver ailments remain one of the serious health problems.

Liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective drugs [20]. The rise in serum levels of SGOT, SGPT, and ALP has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [21]. When rats were treated with CCl_4, it induces hepatotoxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical (CCl_3) which combined with cellular lipids and proteins in the presence of oxygen to induce LPO [22].

Ethanol extract of *H. leschenaultiana* whole plant at the doses 200 and 300 mg/kg significantly restored the elevated levels of serum marker enzymes. The normalization of serum markers by *H. leschenaultiana* whole plant suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl_4 induced leakages of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes.

The administration of CCl_4 alone may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins. The administration of whole plant ethanol extract of *H. leschenaultiana* significantly (p<0.05) reversed these changes may be increasing protein synthesis. This indicates the hepatoprotective activity of ethanol extract *H. leschenaultiana* whole plant against damaged by CCl_4. Increase in serum bilirubin levels may be found in hepatocellular damage, hemolytic jaundice or hepatitis. CCl_4 injury causes significant degeneration of hepatocytes and blockade of the bile ducts which results into significant increase in the serum total bilirubin [23]. The significant reduction of total, conjugated and unconjugated bilirubin levels in the serum when administrated with the ethanol extract of *H. leschenaultiana* whole plant, which indicates that the conjugating function of the liver was improved. The reduction of the bilirubins level by the extracts suggest that the extract may activate the constitutive androstane receptor which is a key regulator in bilirubin clearance in the liver [24].

GGT is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum GGT is generally elevated as a result of liver disease since GGT is a hepatic microsomal enzyme. Serum GGT is most useful in the diagnosis of liver diseases. Changes in GGT is parallel to those of amino transferases. The acute damage caused by CCl_4 increased the GGT level, but the same attains the normal after *H. leschenaultiana* treatment due to its antioxidant activity.

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of antioxidant enzymes such as GPx, GRD, SOD, and CAT. When the balance between ROS production and antioxidant defense is lost, oxidative stress results, which through a series of events deregulates the cellular functions leading to various pathological conditions [25]. Any compound, natural or synthetic, with antioxidant properties might contribute toward the partial or total alleviation of this type of damage.

LPO has been postulated to the destructive process of liver injury due to CCl_4 administration. In this study, the elevations in the levels of end products of LPO in the liver of the rat treated with CCl_4 were observed. The increase in MDA levels in liver suggests enhanced LPO leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Treatment with *H. leschenaultiana* whole plant significantly reversed these changes. Hence, it may be possible that the mechanism of hepatoprotection by ethanol extract of *H. leschenaultiana* whole plant due to its antioxidant effects.

GSH, extensively found in cells, protects cells against electrophilic attacks provided by xenobiotics such as free radicals and peroxides GSH deficiency leads to cellular damage in kidney, muscle, lung, jejunum, colon, liver, lymphocytes, and brain [26]. The elevation of MDA level, which is one of the end products of LPO in the liver tissue, and the reduction in hepatic GSH levels are important indicators in CCl_4 intoxicated rats. In this study, it was ascertained that MAD levels have been suppressed compared to CCl_4 intoxicated group and CCl_4 induced depletion of GSH was prevented.

Table 4: Effect of *H. leschenaultiana* whole plant extract on serum LPO, GPx, GRD, SOD, CAT and GSH activity in the normal control, liver injured and drug treated rats

Groups	Parameters					
	LPO (n mole of MDA/mg protein)	GPX (u/mg protein)	GRD (u/mg)	SOD (u/mg)	CAT (u/mg)	GSH (u/mg)
Group I	2.114±0.089	3.988±0.114	0.504±0.39	0.294±0.019	4.102±0.029	29.94±0.63
Group II	6.169±0.054**	1.956±0.118**	0.311±0.059**	0.093±0.033**	1.845±0.055**	13.63±0.14**
Group III	4.121±0.076*	2.684±0.016^{ns}	0.387±0.031^{ns}	0.188±0.017*	2.261±0.067^{ns}	19.56±0.17^{ns}
Group IV	2.124±0.068^{a}	3.154±0.023	0.451±0.046^{a}	0.219±0.065^{a}	3.541±0.081^{a}	30.63±0.27
Group V	1.948±0.029^{aa}	4.222±0.084^{a}	0.497±0.061^{a}	0.289±0.036^{aa}	4.331±0.094^{aa}	36.59±0.36^{a}
Group VI	2.001±0.014^{a}	4.019±0.079^{a}	0.487±0.059^{a}	0.297±0.014^{a}	4.188±0.076^{aa}	33.91±0.41^{a}

Values are mean±SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *p<0.05; **p<0.01 as compared with normal control to liver damaged control: ^{a}p<0.05; ^{aa}p<0.01 as compared with liver damaged control to drug treated animal. ns: Not significant. SD: Standard deviation, *H. leschenaultiana: Hedyotis leschenaultiana*, LPO: Lipid peroxidation, GPx: Glutathione peroxidase, GRD: Glutathione reductase, SOD: Superoxide dismutase, CAT: Catalase, GSH: Reduced and glutathione, MDA: Melondialdehyde

SOD, a metalloprotein is the most sensitive enzyme index in liver injury and one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to from hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical [27]. In this study, it was observed that the ethanol extract of *H. leschenaultiana* whole plant significantly increased the SOD activity in CCl_4 intoxicated rats thereby diminished CCl_4 induced oxidative damage.

CAT is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found to the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [28]. Therefore, the reduction in the activity of these enzymes may result in the number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide.

Administration of ethanol extract of *H. leschenaultiana* increased the activities of CAT in CCl_4 induced liver damage in rats to prevent the accumulation of excessive free radical and protected the liver from CCl_4 intoxication. GPx is a selenoenzyme and it protects the cells from damage due to free radicals like hydrogen and lipid peroxides [29]. It catalyzes the reaction of hydroperoxidases with reduced glutathione to form glutathione disulfide and reduction.

CONCLUSION

In conclusion, the results of this study suggest that *H. leschenaultiana* whole plant has a potent hepatoprotective action on CCl_4 induced oxidative stress and liver toxicity in rats. The hepatoprotective effect of *H. leschenaultiana* can be correlated directly with its ability to reduce the activity of serum enzymes and enhance antioxidant defense status. The findings of this study suggest that *H. leschenaultiana* can be used as a safe, cheap and effective alternative chemopreventive and protective against in the management of liver diseases.

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