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HEPATOPROTECTIVE ACTIVITY OF THE ETHANOLIC EXTRACT OF *ASTRAEUS HYGROMETRICUS* (PERS.) MORG

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Chronic hepatotoxicity was induced by po introduction of CCl_4 (2.5 ml/kg body weight/day) in paraffin oil. Administration of ethanolic extract of *Astraeus hygrometricus* (150 mg/kg body weight/day) orally protected the CCl_4 mediated elevation of serum transaminase such as glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate tansaminase (GOT) and of serum alkaline phosphatase (ALP), total bilirubin, and direct bilirubin. The hepatic antioxidant status such as superoxide dismutase and catalase activities were reduced in the CCl_4 alone treated animals. Administration of extract to CCl_4 challenge restored the hepatic antioxidant status. The findings thus suggested ethanolic extract of *A. hygrometricus* protects CCl_4 induced chronic hepatotoxicity in mice by restoring the liver antioxidant status.

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Keywords: Astraeus hygrometricus, Carbon tetrachloride, Lipid peroxidation.

1. Introduction

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injuries induced by various hepatotoxins have been recognized as a major toxicological problem for years [1]. Carbon tetrachloride (CCl_4) is a xenobiotic producing hepatotoxicity in human beings and animals [2, 3]. In fact, it has been shown that the trichloromethyl radical formed in the metabolism of CCl_4 via the liver microsomal cytochrome P450 system, reacts rapidly with molecular oxygen to produce trichloromethyl peroxy radicals. These radicals react with unsaturated fatty acids of phospholipids present in cell membranes, initiating lipid peroxidation in liver cells [4]. Hydrogen atoms are removed from unsaturated fatty acids by such radical-created carbon centered lipid radicals [5]. These lipid radicals quickly add molecular oxygen to form lipid peroxyl radicals, which in turn abstract hydrogen atoms from other lipid molecules, thereby propagating the process of lipid peroxidation [6]. Antioxidants play a crucial role in hepatoprotective ability and hence the search for crude drugs of natural origin with this property has become a central focus of study of hepatoprotection today [7].

Mushrooms have been known to be potential source of antioxidants and also capable of strong inhibition of lipid peroxidation [8-11]. *Asteaus hygrometricus* is commonly called as false earthstar, which is a delicious, gastronomic and nutritionally rich mycorhizal macro fungi dominantly found in the forest floor of *Shorea robusta*. Earlier works on *A. hygrometricus* revealed that the ethanolic extract showed potent *in vitro* free radical scavenging activity and inhibition of lipid peroxidation [12]. Here, an attempt has been made to evaluate the hepatoprotective potency against CCl_4 induced hepatic damage in mice of ethanolic extract of *A. hygrometricus*.

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2. Experimental

Sample collection and preparation

Basidiocarp of *A. hygrometricus* was collected from local market and from the sal (*Shorea robusta* G.f.) forests of Bankura and West Midnapore, West Bengal, India. It was identified according to Ramsbottom and Shajahan & Samajpati [13, 14].

Fresh mushrooms were randomly selected into three samples of 150 g each and air-dried in an oven at 40°C for 48 h. Dried powdered mushroom sample was extracted by stirring with 200 ml of ethanol at 30°C for 24 h at 150 rpm and filtering through Whatman No. 4 filter paper. The residue was then extracted twice with another 200 ml of ethanol as described above. The total extract was then rotary evaporated to dryness at 40°C and redissolved in ethanol to a concentration of 10 mg/ml and stored at -20°C for further use [12].

Animals

Swiss albino mice (25–30 g) of approximately the same age were used for the study. They were maintained at a temperature of 25°±3°C and relative humidity of 45% to 55% under 12-h light:12-h dark cycle. Feed and water were supplied *ad libitum*. The animals were maintained according to the guidelines recommended by Animal Welfare Board and approved by our Institutional Animal Ethical Committee (IAEC) constituted under the guidelines of Committee for the Purpose of Control and Supervision on Experimental Animal (CPCSEA), Ministry of Environment, Government of India, New Delhi. All procedures complied with the Declaration of Helsinki, as revised in 1996.

Determination of hepatoprotective effect

Animals were divided into four groups of five animals each. Group I, treated with vehicle (normal saline), was kept as normal (5 ml/kg body wt/day, po). Group II, kept as control, were given CCl_4 in paraffin oil (1:1, v/v, 2.5 ml/kg body wt/day, po). Groups III and IV was treated orally with the ethanolic extract of *A. hygrometricus* and standard reference drug silymarin (150 and 100 mg/kg body wt/day respectively for 7 consecutive days) simultaneously with toxicant (CCl_4/paraffin oil). The animals were sacrificed 24 h after the last treatment of CCl_4. Coagulated blood was collected directly from the heart of each animal. Serum was used for the determination of glutamate pyruvate transaminase (GPT) [15], glutamate oxaloacetate transaminase (GOT) [15], total and direct bilirubin [16] and alkaline phosphatase (ALP) [17]. Liver was removed for the determination of antioxidants and also for histopathological observations.

Evaluation of antioxidants in liver

Liver was removed and washed thoroughly in ice-cold saline, and a homogenate (10%) was prepared in PBS (50mM, pH 7). The homogenate was centrifuged at 5000 × g for 10 min in a centrifuge at 4°C, and after removal of the cell debris, the supernatant was used for the assay of superoxide dismutase (SOD) [18], catalase (CAT) [19], malondialdehyde (MDA) [20] and estimation of reduced glutathione (GSH) [21]. Protein was determined by the method of Lowry *et al.*, 1951 [22].

Histopathological examination

A portion of the liver was fixed in Bouin’s fixative and then embedded in paraffin. Microtome sections of 6 mm thickness were prepared from each liver and stained with hematoxylin-eosin (H&E). The sections were evaluated for the pathological assessment of hepatotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

3. Results

Hepatoprotection

Chronic exposure to CCl_4 elevated the serum GOT and GPT activities (Table 1). The GPT and GOT activities in the CCl_4 alone injected animal were 235.67 ± 35 and 270 ± 40 IU/l, respectively. The activity was significantly lowered by the ethanolic extract of *A. hygrometricus*. The inhibition of activity of the serum transaminases (GPT and GOT) were 56.72% and 54.82%, respectively, compared with the control group animals. Similarly, the serum ALP activity was elevated in the CCl_4-treated control animals. The inhibition of ALP activity by the extract was 30.88% with respect to the control animals. The serum total and direct bilirubin levels were also elevated in the CCl_4 alone injected group. The levels were reduced significantly in the extract-treated group.

Table 1. Effect of ethanolic extract (Ee) of A. hygrometricus serum GOT, GPT, total and direct bilirubin, and ALP activities in mice with chronic CCl_4 administration. Values are mean ± SD, n = 6 animals. GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ALP, alkaline phosphatase.

Groups	Normal	Control (CCl_4/paraffin oil, 1:1)	Ee+CCl_4	Silymarin+CCl_4
Treatment (mg/kg)	Vehicle	—	150	100
Serum GPT (IU/l)	68.5±15	235.67±35	102±11	78±12
Serum GOT (IU/l)	98±26	270±40	122±21	96.4±14
Total bilirubin (mg/dl)	1.219±0.43	2.114±0.38	1.37±0.18	1.296±0.26
Direct bilirubin (mg/dl)	0.131±0.031	0.635±0.088	0.132±0.028	0.139±0.045
Serum ALP (KA)	7.82±0.28	13.13±0.94	9.075±0.57	7.695±0.65

Antioxidant status in liver

The activities of SOD and CAT were decreased in the CCl_4 alone treated group. The activity of SOD in the CCl_4 injected animals was 4.69 ± 0.19 U/mg protein. Treatment of extract (150 mg/kg body wt) enhanced the activity to 6.22 ± 0.34 U/mg protein (Table 2). The activities of CAT in the CCl_4 alone treated animals was 56.7 ± 4.5 U/mg protein which got increased to 65.5 ± 3.4 U/mg protein as a result of extract treatment (Table 2). Results clearly revealed increase in the levels of MDA in CCl_4 intoxicated group, which is significantly diminished on treatment with extract. GSH was also found to be augmented by our extract, which is a sign of improved hepatic function.

Table 2. Effect of ethanolic extract (Ee) of A. hygrometricus on hepatic SOD, CAT, MDA and GSH activities in mice with chronic CCl_4 administration. Values are mean ± SD, n = 6 animals. SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; MDA, malondialdehyde

Groups	Normal	Control (CCl_4/paraffin oil, 1:1)	Ee+CCl_4	Silymarin+CCl_4
Treatment (mg/kg)	Vehicle	—	150	100
SOD (U/mg)	7.42 ± 0.42	4.69 ± 0.19	6.22 ± 0.34	6.98 ± 0.42
CAT (U/mg)	80.7 ± 6.4	56.7 ± 4.5	65.5 ± 3.4	69.4 ± 2.3
GSH (mg/100g)	49.5 ± 5.1	24.7 ± 4.4	35.7 ± 2.5	39.9 ± 4.8
MDA (nmol/mg)	185.4 ± 1.1	477.3 ± 5.6	299.9 ± 4.5	253.4 ± 3.6

Histopathological observations

Histopathological observations showed severe necrosis, fatty infiltration, fibrosis, and lymphocyte infiltration in the hepatocyte of CCl_4 alone treated animals (Figure 1). The effects were moderate to low in the liver of extract and silymarin treated animals showing more or less normal lobular pattern almost comparable to normal set with well-preserved cytoplasm, prominent nucleus and visible central veins.

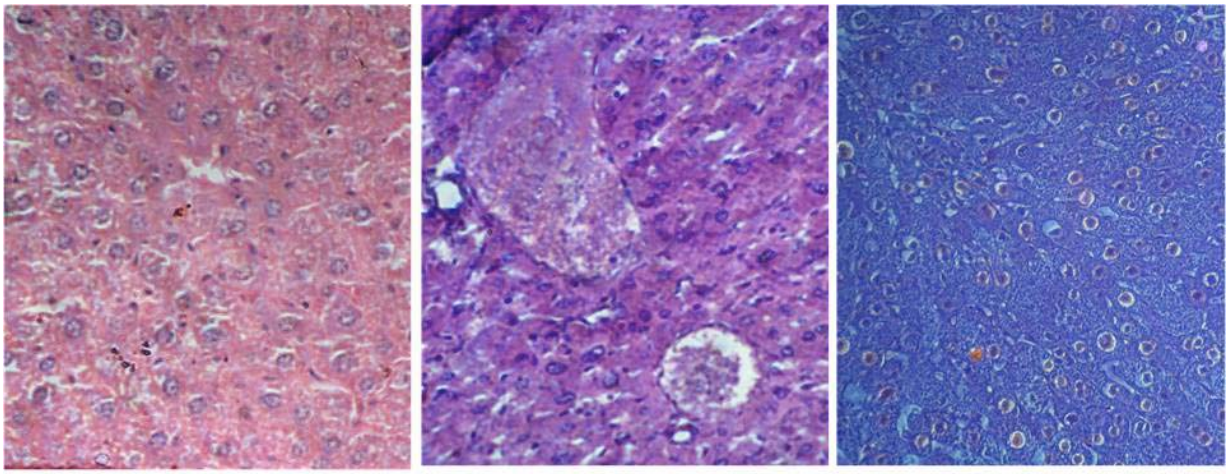

Figure 1. Hepatoprotective activity of ethanolic extract of A. hygrometricus against CCl_4-induced chronic hepatotoxicity in mice. Liver sections stained with H&E.
(a) Normal; (b) CCl_4 treated; (c) Ethanolic extract (150 mg/kg body wt) + CCl_4 treated.

4. Discussion

Results of the current study reveal the hepatoprotective activity of the ethanolic extract of *A. hygrometricus* against chronic hepatotoxicity induced by CCl_4. The activity of transaminases (GPT and GOT) was elevated significantly in the serum of CCl_4 alone injected animals. The elevation of GPT in the serum indicates the necrosis of the hepatocyte, and hence, the altered ratio of GPT to GOT in animals injected with CCl_4. The elevated serum ALP activity was due to the intrahepatic cholestasis, which was reduced in the extract-treated animals. The extract also prevented the elevation of total and direct bilirubin consequent to CCl_4 treatment, indicating the hepatoprotective activity of the extract. The antioxidant status of the hepatocytes was altered in the CCl_4 alone treated animals. The treatment of ethanolic extract of *A. hygrometricus* with CCl_4 effectively protected the decline of antioxidant activity. CCl_4 is metabolized in Cyt P450 system to give the trichloromethyl radical (CCl_3). Trichloromethyl radical reacts with oxygen to form trichloroperoxy radical ($CCl_3O_2^{\cdot}$). Both these products induce the peroxidation of lipids [23]. The products of peroxidation are known to inhibit protein synthesis and activity of certain enzymes. Administration of CCl_4 alone decreased the activity of CAT and SOD in the liver. The declined antioxidant enzyme activity is responsible for the increased lipid peroxidation measured as thiobarbituric acid reacting substance MDA, which leads to loss of membrane fluidity, membrane integrity, and finally loss of cell functions of liver [24, 25]. This may result in the leakage of enzymes, toxic metabolites and free radicals to circulation. The treatment of the extract increased the hepatocyte SOD, CAT activities and reduced lipid proxidation, which could effectively prevent radical mediated loss of membrane integrity. GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals including CCl_4. The significant impairment of hepatic GSH status associated with a substantial hepatocellular damage induced by CCl_4 suggested the determinant role of hepatic GSH in the development of CCl_4 toxicity [26].

The fat accumulation in the liver of CCl_4-treated animals is due to blockage in the synthesis of lipoproteins that carry triacylglycerol away from the liver. Histopathological observation of the CCl_4 and CCl_4 plus extract treated liver clearly shows the differential level of fatty infiltration and necrosis due to radical-mediated cytotoxicity. The direct radical-scavenging activity of the extract might be involved in the hepatoprotective activity against chronic CCl_4 exposure. The results of the current study indicate that the hepatoprotective effect of ethanolic extract of *A. hygrometricus* is mediated through the antioxidant defense mechanism.

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