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Research Article

ANTIHEPATOTOXIC INFLUENCE OF AQUEOUS EXTRACT OF *IPOMOEA CARNEA* AGAINST CARBON TETRACHLORIDE INDUCED ACUTE LIVER TOXICITY IN EXPERIMENTAL RODENTS

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ABSTRACT

The present study was undertaken to investigate the hepatoprotective potential of *Ipomoea carnea* leaves in experimental rats. The aqueous extract of *Ipomoea carnea* leaves (ICAE, 125, 250 and 500 mg/kg body weight) was administered daily for 14 days in experimental animals. Liver injury was induced chemically, by CCl_4 administration (1 ml/kg *i.p.*). The hepatoprotective activity was assessed using various biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), Serum alkaline phosphatise (SALP) and total bilirubin. Meanwhile, *in vivo* antioxidant activities as lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were screened along with histopathological studies. Obtained results demonstrated that the treatment with ICAE significantly ($P<0.05$-$P<0.001$) and dose-dependently prevented chemically induced increase in serum levels of hepatic enzymes. Furthermore, ICAE significantly (up to $P<0.001$) reduced the lipid peroxidation in the liver tissue and restored activities of defense antioxidant enzymes GSH, SOD and catalase towards normal levels. Histopathology of the liver tissue showed that ICAE attenuated the hepatocellular necrosis and led to reduction of inflammatory cells infiltration. The results of this study strongly indicate the protective effect of ICAE against acute liver injury which may be attributed to its hepatoprotective activity.

Keywords: *Ipomoea carnea* aqueous extract (ICAE), Hepatoprotective, CCl_4, Antioxidant, and Aspartate-aminotransferase.

INTRODUCTION

Liver diseases are one of the most serious health problems in the world today but, despite tremendous advances in modern medicine, their prevention and treatment options still remain limited. However, the pathogenesis of hepatic diseases as well as the role of oxidative stress and inflammation therein is well established[1,2], and accordingly, blocking or retarding the chain reactions of oxidation and inflammation process could be a promising therapeutic strategy for prevention and treatment of liver injury. Detoxification reactions (phase I and phase II) metabolize xenobiotics with the aim of increasing substrate hydrophilicity for excretion. Drug-metabolizing enzymes detoxify many xenobiotics but bioactivate or increase the toxicity of others. In the case of bioactivation, liver is the first organ to be exposed to the damaging effects of the newly formed toxic substance. Therefore, protective mechanisms relevant to the liver are of particular interest. Effectively, herbal products are widely used in the treatment of hepatic disorders all over the world[3]. The plant *Ipomoea carnea* is a large, diffuse or struggling shrub with milky juice, leaf ovate cordate, entire, acuminate, flower large campanulate, pale rose, pink or light violet in lax, dichotomously branched axillary and terminal, pedunculate cymes; Fruits glabrous capsule; Seed silky, belonging to family Convolvulaceae [4,5,6.] It is well distributed in India and found particularly in Chhattisgarh and Madhya Pradesh [7,8,9].The plant is commonly known as Besharam, Behaya and used for skin troubles successfully. The milky juice of Beshram is used for the treatment of leucoderma[10].The juice is collected and applied externally on affected parts, anti-inflammatory. It is used to decrease the teratogenic effect resulting from cyclophosphamide[11]. Aqueous extract of *Ipomoea carnea* shows neuromuscular blocking activity[12]. It used as aphrodisiac, purgative and cathartic[13]. The leaves of *Ipomoea carnea* contain 1-3 flavonol glycosides and Ergine (D-Lysergic acid amide)[14]. Polyhydroxylated alkaloids were isolated from the leaves, flowers and seeds[15]. Chromatographic separation of the leaf extract resulted in the isolation of swainsonine, 2-epilentiginosine, calystegines B (1), B (2), B (3) and C (1) and N-methyl-trans-4-hydroxy-l-proline and beta sitosterol [16,17,18].

MATERIALS AND METHODS

Plant material

The plant *Ipomoea carnea* is widely found throughout India. The plant herbarium specimen was identified and authenticated by Mr. P. G. Diwakar, Joint Director, Botanical Survey of India, Western circle-7, Koregaon Road, Pune -1 on dated 11/01/2011, Voucher No. RASICA4. The leaves were dried in shade at room temperature. The dried leaves were coarsely powdered, stored in airtight container until used and packed in soxhlet apparatus. Extraction of leaves of *Ipomoea carnea* was carried out by using soxhlet apparatus. Polar solvent petroleum ether, chloroform, ethanol and water were used according to the polarity.

Experimental animals

Healthy albino Wistar rats of age between 10-15 weeks of either sex were used after approval of the institutional ethics committee. They were kept in departmental animal house in well cross ventilated room at 22 ± 2 °C with light and dark cycles of 12 h for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was given *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee.

Acute toxicity study

Acute toxicity study was performed according to OECD guidelines No. 420. Swiss albino mice of either sex were divided into six groups with six animals each. Aqueous extracts of *Ipomoea carnea* leaves were studied for acute toxicity at doses different dose levels of 5, 50, 300, 500 and 2000 mg/kg b.w. Animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 days[19].

CCl_4 induced hepatotoxicity

The animals were divided into six groups, each group had six animals. Group I (control) animals were administered a single daily dose of carboxymethyl cellulose (1 ml of 1%, w/v, *p.o.* body weight). Group II received carbon tetrachloride (1 ml/kg body weight, *i.p.* 1:1 v/v mixture of CCI_4 and liquid paraffin) alone while group III, IV and V received orally 125, 250 and 500 mg/kg body weight of ICAE in (1%, w/v, CMC) respectively along with carbon tetrachloride as in group II. Group VI received silymarin, the known hepatoprotective compound (Sigma Chemicals Company, USA), at a dose of 100 mg/kg, *p.o.*, along with carbon tetrachloride. The ICAE was given daily while carbon tetrachloride was given for every 72 h for 14 days. Animals were sacrificed 48 h after the last dose of the drug. The liver samples were dissected and blood was collected[20].

Assessment of hepatoprotective activity

The collected blood was allowed to clot and serum was separated at 2500 rpm for 15 min and the biochemical parameters like serum enzymes: aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L)[21], serum alkaline phosphatase (ALP, U/L)[22] and total bilirubin (mg/dL)[23] were assayed using assay kits.

Assessment of antioxidant parameters

The dissected out liver samples were washed immediately with ice cold saline to remove as much blood as possible. Liver homogenized (5%) in ice cold 0.9% NaCl with a Potter- Elvenhjem glass homogenizer. The homogenate was centrifuged at 800 for 10 min and the supernatant was again centrifuged at 12,000 for 15 min and the obtained mitochondrial fraction was used for the estimation of LPO[24], catalase (CAT)[25]. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate–nitrobluetetrazolium reaction system[26]and GSH[27].

Histopathological studies

For histopathological studies, the liver tissues were fixed with 10% phosphate buffered neutral formalin, dehydrated in graded (50–100%) alcohol and embedded in paraffin. Thin sections (5 µM) were cut and stained with routine hematoxylin and eosin stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

Statistical analysis

The values were represented as mean ± S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman-Keuls test using Prism Pad software (Version-5) for the determination of level of significance. The values of $p<0.05$ was considered statistically significant.

RESULTS

Acute toxicity studies

As per OECD 420 guideline dose of 2000mg/kg showed the toxic symptoms, so according to OECD guideline 420, it is considered as a LD-50 cut-off value. Doses selected for pharmacological studies by fixed dose methods are 125mg/kg, 250 mg/kg and 500mg/kg body weight. Thus three doses (125, 250 and 500 mg/kg p.o.) were employed for further pharmacological studies.

Effect of ICAE on AST, ALT, ALP and total bilirubin

The effect various doses of ICAE were studied on serum marker enzymes and total bilirubin in CCl_4 intoxicated animal. Hepatic injury induced by CCl_4 caused significant changed in marker enzyme as AST by 278.67%, ALT by 386.79%, ALP by 135.24% and total bilirubin by 310.24% compared to control group. The percentage protection in marker enzyme of treated group at 125, 250 mg/kg as AST 28.71 (P< 0.01), 51.17 (P< 0.001), ALT 25.76 (P< 0.05), 54.43 (P< 0.001), ALP 17.94 (P< 0.05), 41.22 (P< 0.001) and total bilirubin 28.52 (P< 0.01), 62.82 (P< 0.001) compared to CCl_4 group while maximum percentage protection in marker enzyme at the dose of 500 mg/kg and silymarin (100mg/kg) as AST 68.08 (P< 0.001), 70.23 (P< 0.001), ALT 75.80 (P< 0.001), 77.49 (P< 0.001), ALP 48.80 (P< 0.001), 54.78 (P< 0.001) and total bilirubin 72.43 (P< 0.001), 74.01 (P< 0.001) which is almost comparable to the group treated with silymarin, a potent hepatoprotective drug used as reference standard (Table 1).

Table 1: Effect of ICAE on serum GOT (U/L), GPT (U/L), ALP (U/L), Bilirubin level (mg/dl) and liver LPO (MDA nmole/min/mg of protein), SOD (unit/mg of protein), CAT (units/mg of protein) and GSH (nmole/mg of protein) against CCl_4 induced liver toxicity in rats.

Groups	AST	ALT	ALP	TBL	LPO	GSH	SOD	CAT
Control	100.26 ± 18.13	47.71 ± 8.21	66.13 ± 7.88	0.76 ± 0.15	0.38 ± 0.08	0.81 ± 0.07	28.23 ± 2.82	55.51 ± 5.52
CCl_4	379.66 ± 36.21†	232.25 ± 24.77†	155.57 ± 14.55†	3.12 ± 0.39†	1.37 ± 0.17†	0.34 ± 0.04†	9.23 ± 1.6†	34.41 ± 2.37†
ICAE 125	270.65 ± 23.59^{b}	172.41 ± 18.24^{a}	127.66 ± 11.21^{b}	2.23 ± 0.14^{b}	0.93 ± 0.13^{a}	0.50 ± 0.05^{a}	14.25 ± 1.22^{a}	39.52 ± 2.57^{n}
ICAE 250	185.63 ± 19.80^{c}	105.83 ± 14.46^{c}	91.43 ± 8.61^{c}	1.16 ± 0.11^{c}	0.63 ± 0.17^{b}	0.66 ± 0.08^{b}	19.41 ± 2.6^{c}	46.45 ± 3.11^{a}
ICAE 500	121.17 ± 16.55^{c}	56.17 ± 9.33^{c}	79.65 ± 7.54^{c}	0.86 ± 0.13^{c}	0.43 ± 0.12^{c}	0.73 ± 0.05^{c}	23.61 ± 2.5^{c}	52.4 ± 3.45^{b}
Silymarin	112.99 ± 12.41^{c}	52.26 ± 6.72^{c}	70.34 ± 7.11^{c}	0.81 ± 0.12^{c}	0.40 ± 0.09^{c}	0.80 ± 0.06^{c}	24.32 ± 2.3^{c}	54.1 ± 2.41^{b}

Values are mean ± S.E.M. of 6 rats in each groupn : non significantP values: †<0.001 compared with respective control group IP values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 compared with group II (CCl_4)

Estimation of LPO, GSH, SOD and CAT

The results in table 1 showed clear significant percentage change in the levels of LPO in CCl_4 intoxicated rats as 260.52 (P< 0.001) compared to control group. Treatment with ICAE at the doses of 125, 250 and 500 mg/kg significantly prevented this heave in levels and the percentage protection in LPO were 32.11 (P< 0.05), 54.01 (P< 0.01) and 68.61 (P< 0.001) respectively. The GSH, SOD and CAT content had significantly increased in ICAE treated groups whereas CCl_4 intoxicated group had shown significant decrease in these parameters compared to control group.

The percentage changed of GSH, SOD and CAT in CCl_4 intoxicated group were as 58.02 (P< 0.001), 67.30 (P< 0.001) and 34.41 (P< 0.001) respectively.

The percentage protection in GSH as 47.05 (P< 0.05), 94.11 (P< 0.01), 114.70 (P< 0.001) and SOD 54.38 (P< 0.05), 110.29 (P < 0.001), 155.79 (P < 0.001) while in CAT 14.86 (ns), 34.98 (P<0.05), 52.21 (P< 0.01) at the doses levels 125, 250 and 500 mg/kg, respectively. In different doses level of ICAE, 500 mg/kg has shown maximum protections which were almost comparable to those of the normal control and silymarin.

Histopathological observations

The histological observations basically support the results obtained from serum enzyme assays. Histopathology of liver section is well described in figure 1 ligand.

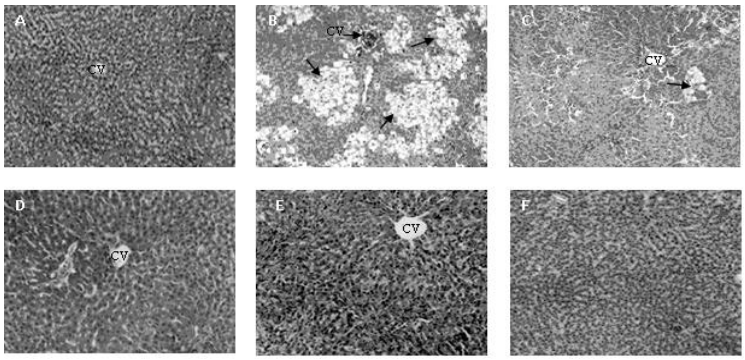

Figure 1: Histopathology of liver tissues: A- Liver section of normal control rat shows central vein surrounded by hepatic cord of cells i.e. normal architecture. B- Liver section of CCl_4 treated rats showing massive fatty changes along with congestion in central vein, necrosis, sinusoidal dilation, ballooning degeneration and the loss of cellular boundaries. C- Liver section of rats treated CCl_4 and 125 mg/kg of ICAE showing inflammatory collections around central vein, less ballooning and focal necrosis with sinusoidal dilatation. D- Liver section of rats treated CCl_4 and 250 mg/kg of IPAE showing less inflammatory cells around central vein, absence of necrosis. E-Liver section of rats treated CCl_4 and 500 mg/kg of ICAE showing regeneration of hepatocytes around central vein toward near normal liver. F- Liver section of rats treated CCl_4 and 100 mg/kg of silymarin showing normal liver architecture.

DISCUSSION

In the present investigation, *Ipomoea carnea* (ICAE) was evaluated for the hepatoprotective activity using CCl_4 induced hepatotoxicity in rat. The hepatotoxicity induced by CCl_4 is due to its metabolite $CCl_3^{\bullet}$, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage[28]. Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into blood[29].

The present study revealed a significant increase in the activities of AST, ALT, ALP and serum bilirubin levels on exposure to CCl_4, indicating considerable hepatocellular injury. Administration of ICAQ at different doses level (125, 250 and 500 mg/kg) attenuated the increased levels of the serum enzymes, produced by CCl_4 and caused a subsequent recovery towards normalization comparable to the control groups animals. The hepatoprotective effect of the ICAE was further accomplished by the histopathological examinations. ICAE at different dose levels offers hepatoprotection, but 500 mg/kg is more effective than all other lower doses.

In CCl_4 induced hepatotoxicity, the balance between ROS production and these antioxidant defences may be lost, 'oxidative stress' results, which through a series of events deregulates the cellular functions leading to hepatic necrosis. The reduced activities of SOD and catalase observed point out the hepatic damage in the rats administered with CCl_4 but the treated with 125, 250 and 500 mg/kg of ICAE groups showed significant increase in the level of these enzymes, which indicates the antioxidant activity of the *Ipomoea carnea*. Regarding non enzymic antioxidants, 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+. Furthermore, a decrease in hepatic tissue GSH level was observed in the CCl_4 treated groups. The increase in hepatic GSH level in the rats treated with 125, 250 and 500 mg/kg of ICAE may be due to de novo GSH synthesis or GSH regeneration. The level of lipid peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, elevation of lipid peroxidation in the liver of rats treated with CCl_4 was observed. The increase in LPO levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals[31]. Treatment with ICAE significantly reversed all the changes. Hence, it is possible that the mechanism of hepatoprotection of *Ipomoea carnea* may be due to its antioxidant activity.

On phytochemical screening, ICAE revealed the presence of alkaloids, tannins, and flavonoids are the major chemical constituents. These phytochemical might contribute to the hepatoprotective and antioxidant activities of the ICAE.

CONCLUSION

From the results it is concluded that the ICAE has shown dose dependent activity among which at the dose level of 500 mg/kg, *p.o.* shows greater activity which is comparable with the control and standard groups. However, further investigation is in process on the leaf extract to identify the active constituents responsible for hepatoprotection.

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