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Anti−hepatotoxic and antioxidant influence of *Ipomoea carnea* against anti−tubercular drugs induced acute hepatopathy in experimental rodents

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PEER REVIEW

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Comments

This is a unique work by which the authors evaluated anti−hepatotoxic and antioxidant influence of *I. carnea* against anti−tubercular drugs induced acute hepatopathy. The *I. carnea* extracts showed significant results compared with the standard.
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ABSTRACT

Objective: To assess the hepatoprotective effect of *Ipomoea carnea* (*I. carnea*) extract against antitubercular drug−induced liver toxicity in experimental animals.
Methods: *I. carnea* extracts (125, 250 and 500 mg/kg, *p.o.* body weight) were administered daily for 35 d in experimental animals. Liver toxicity was induced by combination of three antitubercular drugs (isoniazid 7.5 mg/kg, rifampicin 10 mg/kg and pyrazinamide 35 mg/kg) given orally as suspension for 35 d in rats. Treatment groups received *I. carnea* extracts along with antitubercular drugs. The hepatoprotective activity was assessed using various biochemical parameters like aspartate aminotransferase, alanine aminotransferase, alkaline phosphatise and total bilirubin. Meanwhile, *in−vivo* antioxidant activities as lipid peroxidation, reduced glutathione, superoxide dismutase and catalase were measured in rat liver homogenate along with ATPase and G−6−Pase. The biochemical observations were supplemented by histopathological examination.
Results: Obtained results demonstrated that treatment with *I. carnea* extracts significantly (P<0.05−P<0.001) and dose−dependently prevented drug induced increase in serum levels of hepatic enzymes. Furthermore, *I. carnea* extracts significantly (up to P<0.001) reduced the lipid peroxidation in the liver tissue and restored activities of defence antioxidant enzymes, reduced glutathione, superoxide dismutase and catalase towards normal levels. Histopathology of the liver tissue showed that *I. carnea* extracts attenuated the hepatocellular necrosis, massive fatty changes and led to reduction in inflammatory cells infiltration.
Conclusions: The results of this study strongly indicate the protective effect of *I. carnea* extracts against liver injury, which may be attributed to its hepatoprotective activity, and there by scientifically support its traditional use.

KEYWORDS
Ipomoea carnea, Hepatoprotective, Antioxidant, Rifampicin, Isoniazid, Pyrazinamide

1. Introduction

Today, human beings are exposed on a daily basis to certain environmental pollutants and foreign chemicals collectively referred as xenobiotics which are causing serious health problems. Liver diseases have become a global problem and about 20000 deaths occur every year due to liver disorders[1]. Severe liver diseases are characterized by a progressive

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evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, hepatocellular carcinoma and their prevention and treatment options still remain limited. There is increasing evidence that free radicals and reactive oxygen species play a crucial role in various steps that initiate and regulate the progression of liver diseases independent of the agent of origin[2,3]. Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress[4]. 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[5]. Therefore, protective mechanisms relevant to the liver are of particular interest. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices and in traditional system of medicine in India[6]. Indian medicinal plants belonging to about 40 families have been being investigated as liver protective drugs[7]. Plant flavonoids are antioxidants that can protect liver from oxidative damages[8,9]. Anti tubercular drugs are the commonest agents causing serious, clinically significant acute liver failure in India. Most commonly used anti tubercular drugs like isoniazid, rifampicin and pyrazinamide are hepatotoxic[10]. The plant *Ipomoea carnea* (*I. 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[11]. It is well distributed in India and found particularly in Chhattisgarh and Madhya Pradesh[12]. The plant is commonly known as Besharam, Behaya and used for treating skin troubles. The milky juice of the plant is used for the treatment of leucoderma[13]. The juice is collected and applied externally on affected parts. It also possesses anti-inflammatory effects. It is used to decrease the teratogenic effect resulting from cyclophosphamide[14]. Aqueous extract of *I. carnea* shows neuromuscular blocking activity, aphrodisiac, purgative and cathartic and the leaves of *I. carnea* contain 1-3 flavonol glycosides and ergine (d-lysergic acid amide). Polyhydroxylated alkaloids were isolated from the leaves, flowers and seeds. Chromatographic separation of the leaf extract resulted in the isolation of swainsonine, 2-epilentiginosine, calystegines B1, B2, B3 and C1, N-methyl-trans-4-hydroxy-l-proline and beta sitosterol[15]. To the best of our knowledge, there was lack of scientific reports available in support of its traditional claim of hepatoprotective potential. So far, there has been only one research reported on its hepatoprotective effect against carbon tetrachloride induced liver damage in rats[16]. Therefore, present study was designed to demonstrate the anti-hepatotoxic and antioxidant potential of *I. carnea* against anti-tubercular drugs induced acute hepatopathy in experimental animals.

2. Material and methods

2.1. Drugs and chemicals

All the chemicals used were of analytical grade and procured from Sigma Chemicals Co., USA and Qualigens Fine, Mumbai, India.

2.2. Plant material

The plant *I. carnea* is widely distributed throughout India. Its 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 11/01/2011 (Voucher No. RASICA4). The leaves of *I. carnea* were washed thoroughly in tap water, shade dried and powdered. Powdered material was subjected to extraction in a Soxhlet apparatus at 60-70 °C for 6 h continuously in 50% distilled ethanol. The extracted material was evaporated to dryness under reduced pressure (40-45 °C). The yield of the material was 12.63 g. This crude extract was referred to as *I. carnea* extracts. The extract obtained was further subjected to pharmacological investigation.

2.3. 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 (Reg. No-346/CPCSEA).

2.4. 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 *I. carnea* leaves were studied for acute toxicity at different dose levels of 5, 50, 300, 500 and 2000 mg/kg body weight. Animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 d[16].

2.5. Anti tubercular drugs induced hepatotoxicity

The animals were divided into six groups with each group consisting of six animals. The animals were assigned to different groups according to a block permuted randomization plan. Group I received distilled water and served as healthy control. Group II rats were administered a combination of three anti-tubercular drugs (isoniazid-7.5 mg/kg, rifampicin-10 mg/kg and pyrazinamide-35 mg/kg for 35 d by intra-gASTric administration) in sterile saline which severed as disease control. Groups III, IV and V were administered three graded doses of *I. carnea* extracts *i.e.* 125, 250 and 500 mg/kg, *p.o.* 45 min prior to anti tubercular drugs challenge for 35 d. Group VI received silymarin (as a standard, 100 mg/kg, orally daily) for 35 d, 45 min prior to ATT challenge as a reference[17]. On completion of the experimental period the blood was collected, the animals were sacrificed and liver samples were collected.

2.6. Assessment of hepatoprotective activity

The collected blood was allowed to clot and serum was separated at 2500 r/min for 15 min and serum obtained was frozen at -20 °C until analysis. The biochemical parameters like

aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L)[18], alkaline phosphatase (ALP, U/L)[19] and total bilirubin (TBL, mg/dL)[20], were assayed using span diagnostics assay kits.

2.7. Assessment of tissue biochemical assay

2.7.1. Assessment of lipid peroxidation (LPO)

The dissected liver samples were washed immediately with ice cold saline to remove as much blood as possible. Liver was homogenized (5%) in ice cold 0.9% NaCl with a Potter-Elvehjem glass homogenizer. The homogenate was centrifuged at 800 for 10 min and the supernatant was again centrifuged at 12000 for 15 min and the obtained mitochondrial fraction was used for the estimation of LPO[21]. A volume of the homogenate (0.2 mL) was transferred to a vial and was mixed with 0.2 mL of a 8.1% (w/v) sodium dodecyl sulphate solution, 1.5 mL of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.5 mL of a 0.8% (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to 4.0 mL with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10% TBA were transferred into a centrifuge tube and centrifuged at 1000 *g* for 10 min.

The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrometer). Control experiment was processed using the same experimental procedure except that the TBA solution was replaced with distilled water[22]. Malonyldialdehyde is an end product of lipid peroxidation, which reacts with TBA to form pink chromogen TBA reactive substance. 1, 1, 3, 3-tetra ethoxypropan was used as standard for calibration of the curve and is expressed as nmole/mg protein.

2.7.2. Assessment of reduced glutathione (GSH) activity

The concentration of GSH was determined by the method of Anderson, based on the development of a yellow colour when 5,5-dithiobis (2-nitrobenzoic acid) is added to compounds containing sulfhydryl groups[23]. The reaction mixture contained equal volumes of 4% sulfosalicylic acid and tissue samples homogenized in 4 volume of ice cold 0.1 mol/L phosphate buffer (pH 7.4). The method used for estimating GSH in this study also measured non-protein sulfhydryl concentration inclusive of GSH. However, 80-90% of the non-protein sulfhydryl content of the cell represents free endogenous GSH. Enzyme activity was expressed as milligram per hundred grams.

2.7.3. Assessment of catalase (CAT) and superoxide

The liver tissue was homogenized (5%) and mitochondrial fraction was prepared as described above. Decomposition of H_2O_2 in presence of CAT was followed at 240 nm[24]. One unit of CAT was defined as the amount of enzyme required to decompose 1 μmol of H_2O_2 per min, at 25 °C and pH 7.0. Results are expressed as units of CAT activity/mg protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide reduced-phenazine methosulphate-nitroblue tetrazolium reaction system as described by Nishikimi *et al.*[25] and as adapted by Kakkar *et al*[26]. One unit of the enzyme is equivalent to 50% inhibition in the formazan formation in 1 min at room temperature (25±2) °C and the results have been expressed as units of SOD activity/mg protein.

2.7.4. Assessment of adenosine triphosphatase (ATPase) and glucose-6-phosphatase (G-6-Pase) activity

The activities of ATPase and G-6-Pase were also determined in liver[27,28].

2.8. 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.

2.9. Statistical analysis

The values were represented as mean±SEM for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman-Keuls test using Prism Pad software (version 3.0) for the determination of level of significance. The values of $P<0.05$ was considered statistically significant.

3. Results

3.1. Acute toxicity studies

As per OECD 420 guideline dose of 2000 mg/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 125, 250 and 500 mg/kg body weight. Thus three doses (125, 250 and 500 mg/kg *p.o.*) were employed for further pharmacological studies.

3.2. Effect of I. carnea extracts on serum AST, ALT, ALP, and TBL level

The effect of various doses of *I. carnea* extracts was studied on serum marker enzymes and TBL in antitubercular drugs intoxicated animals. Hepatic injury induced by antitubercular drugs caused significant changes in marker enzyme AST by 196%, ALT by 245.56%, ALP by 199.61% and TBL by 311.84% when compared to control group. The percentage protection in marker enzyme of treated groups at 125, 250 mg/kg as AST 9.04 ($P<0.05$), 18.04 ($P<0.01$), ALT 3.12 (ns), 10.46 ($P<0.05$), ALP 11.09 ($P<0.05$), 19.06 ($P<0.01$), and TBL 16.61 ($P<0.05$), 23.00 ($P<0.01$) when compared to toxic group while maximum percentage protection in marker enzyme was at the dose of 500 mg/kg and silymarin 100 mg/kg as AST 40.33 ($P<0.001$), 48.21 ($P<0.001$), ALT 33.28 ($P<0.001$), 53.66 ($P<0.001$), ALP 43.24 ($P<0.001$), 55.41 ($P<0.001$), TBL 48.24 ($P<0.001$), 67.41($P<0.001$) which is almost comparable to the group treated with silymarin 100 mg/kg, a potent hepatoprotective drug used as reference standard (Table 1).

3.3. Estimation of LPO, GSH, SOD, CAT, ATPase and G-6-Pase

The results in Table 2 shows clear significant percentage change in the antioxidant levels of LPO in antitubercular drugs intoxicated rats as 278.12 ($P<0.001$) compared to control group. Treatment with *I. carnea* extracts at the doses of 125, 250 and

500 mg/kg significantly prevented this heave in levels and the percentage protection in LPO were 14.04 (P<0.05), 19.83 (P<0.01) and 47.93 (P<0.001) respectively. The GSH, SOD, CAT, ATPase and G–6–Pase content had significantly increased in *I. carnea* extracts treated groups where as antitubercular drugs intoxicated group had shown significant decrease in these parameters compared to control group.

Table 1

Effect of *I. carnea* extracts on serum AST, ALT , ALP, and TBL level against R+I+P induced hepatopathy in rats.

Treatments	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TBL (mg/dL)
Control (DW)	58.20±4.47	42.60±3.90	54.14±3.88	0.76±0.18
R+I+P	172.28±9.20†	147.21±5.01†	162.21±8.20†	3.13±0.14†
ICE–125+(R+I+P)	156.70±7.20^{a}	142.61±4.80^{n}	144.21±7.10^{a}	2.61±0.12^{a}
ICE–250+(R+I+P)	141.20±7.10^{b}	131.80±4.20^{a}	131.28±6.20^{b}	2.41±0.16^{b}
ICE–500+(R+I+P)	102.79±6.40^{c}	98.21±3.90^{c}	92.08±4.90^{c}	1.62±0.17^{c}
SYL–100+(R+I+P)	89.21±5.80^{c}	68.21±2.90^{c}	72.32±3.20^{c}	1.02±0.11^{c}

Values are mean±SEM of 6 rats in each group.

ICE: *I. carnea* extracts, DW: distilled water, R: Rifampicin, I: Isoniazid, P: Pyrazinamide. n: non significant; †: P<0.001 compared with respective control group I; ^{a}: P<0.05, ^{b}: P<0.01, ^{c}: P<0.001 compared with group II (R+I+P).

The percentage changes of GSH, SOD, CAT, ATPase and G–6–Pase in antitubercular drugs intoxicated group were 45.08 (P<0.001), 47.24 (P<0.001), 36.55 (P<0.001), 57.08 (P<0.001) and 46.36 (P<0.001) respectively. Table 2 explained the percentage protection in GSH as 39.88 (P<0.05), 46.92 (P<0.01), 74.78 (P<0.001), SOD 7.39 (ns), 21.28 (P<0.05), 46.19 (P<0.001), CAT 6.71 (ns), 18.52 (P<0.05), 32.12 (P<0.01) and ATPase 10.28 (ns), 42.25 (P<0.01), 74.92 (P<0.001) while in G–6–Pase 22.08 (P<0.05), 32.80 (P<0.01), 57.09 (P<0.001) at the doses levels 125, 250 and 500 mg/kg respectively. In different doses levels of *I. carnea* extracts 500 mg/kg has shown maximum protection which was almost comparable to those of the normal control and standard.

3.4. Histopathological observations

The histological observations basically support the results obtained from serum enzyme assays. Liver section in normal control rats showed central vein surrounded by hepatic cord of cells while antitubercular drugs treated rats liver section showed massive fatty changes, focal central vein congestion, necrosis with portal inflammation and loss of cellular boundaries. Liver section of rats treated with antitubercular drugs and 125 mg/kg of *I. carnea* extracts showing mild central vein congestion, necrosis with sinusoidal dilatation. Liver section of rats treated with antitubercular drugs and 250 mg/kg of *I. carnea* extracts showing less inflammatory cells, less hepatic necrosis and absence of central vein congestion while the liver section of rats treated with antitubercular drugs and 500 mg/kg of *I. carnea* extracts showing regeneration of hepatocytes around central vein toward near normal liver architecture possessing higher hepatoprotective action (Figure 1).

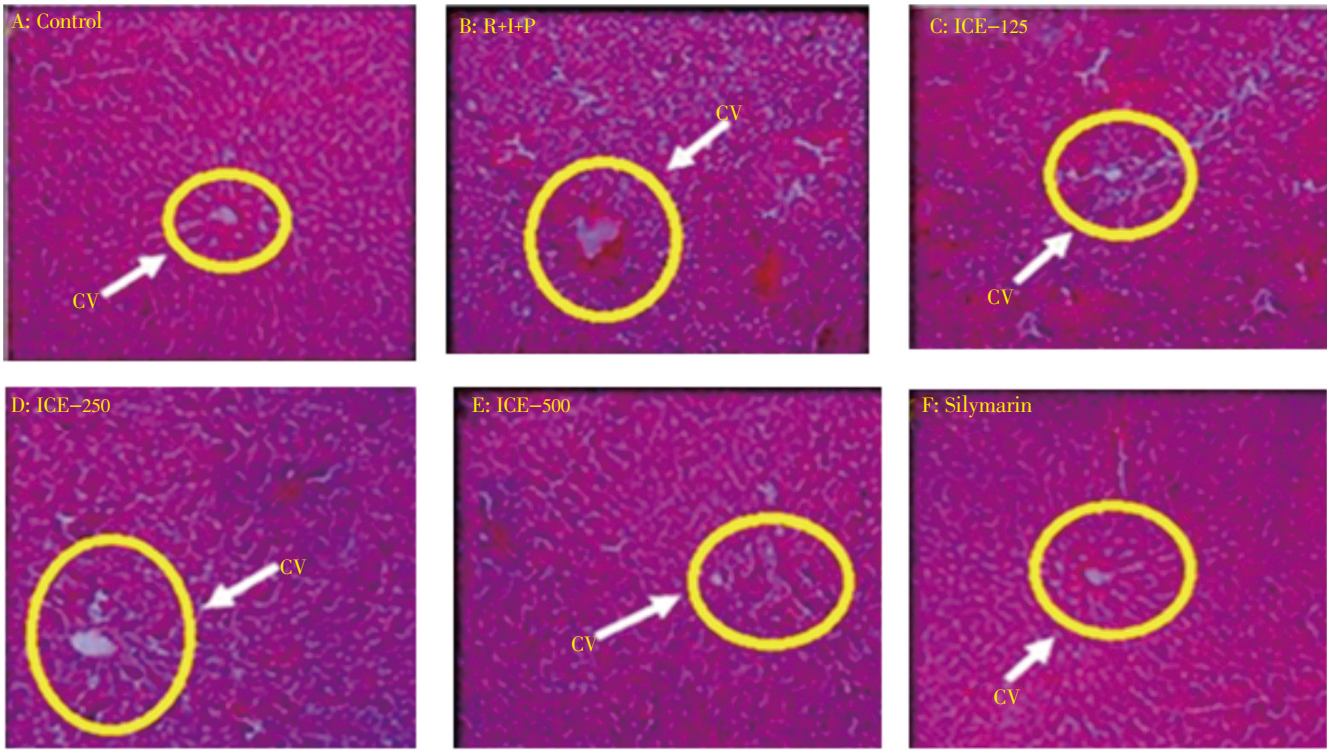

Figure 1. Histopathology of liver tissues.

ICE: *I. carnea* extracts, CV: central vein. A: Liver section of normal control rat shows central vein surrounded by hepatic cord of cells (normal architecture). B: Liver section of antitubercular drugs treated rats showing fatty changes, focal central vein congestion (indicated by arrow), necrosis with portal inflammation and loss of cellular boundaries. C: Liver section of rats treated with antitubercular drugs and 125 mg/kg of *I. carnea* extracts showing mild central vein congestion (indicated by arrow), necrosis with sinusoidal dilatation. (D) Liver section of rats treated with antitubercular drugs and 250 mg/kg of *I. carnea extracts* showing less inflammatory cells, less hepatic necrosis and absence of central vein congestion. (E) Liver section of rats treated with antitubercular drugs and 500 mg/kg of *I. carnea extracts* showing regeneration of hepatocytes around central vein toward near normal liver architecture. (F) Liver section of rats treated with antitubercular drugs and 100 mg/kg of silymarin showing normal liver architecture.

Table 2

Effect of *I. carnea* extracts on liver LPO, GSH, SOD, CAT, ATPase and G-6-Pase against R_4I_4P induced liver toxicity in rats.

Treatments	LPO (MDA nmole/min/mg of protein)	GSH (nmole/mg of protein)	SOD (unit/mg of protein)	CAT (units/mg of protein)	ATPase (mg Pi/100 mL/min)	G-6-Pase (μmole Pi/min/g liver)
Control (DW)	0.32±0.02	6.21±0.21	24.13±2.72	49.51±4.52	1489.21±98.10	5.91±0.26
R+I+P	1.21±0.07†	3.41±0.28†	12.73±1.42†	31.41±2.37†	627.80±52.40†	3.17±0.15†
ICE-125+(R+I+P)	1.04±0.06^{a}	4.77±0.31^{a}	13.21±1.01^{n}	33.52±2.47^{n}	692.37±56.21^{n}	3.87±0.16^{a}
ICE-250+(R+I+P)	0.97±0.05^{b}	5.01±0.32^{b}	15.44±2.23^{a}	37.23±3.21^{a}	886.81±64.21^{b}	4.21±0.20^{b}
ICE-500+(R+I+P)	0.63±0.05^{c}	5.96 ±0.39^{c}	18.61±2.11^{c}	41.50±3.25^{c}	1098.20±72.40^{c}	4.98±0.21^{c}
SYL-100+(R+I+P)	0.48±0.03^{c}	6.03±0.41^{c}	21.32±2.12^{c}	44.80±2.21^{c}	1237.72±89.60^{c}	5.71±0.31^{c}

ICE: *I. carnea* extracts, DW: distilled water, R: Rifampicin, I: Isoniazid, P: Pyrazinamide. n: non significant; †: $P<0.001$ compared with respective control group I; ^{a}: $P<0.05$, ^{b}: $P<0.01$, ^{c}: $P<0.001$ compared with group II (R+I+P).

4. Discussion

In the present investigation, *I. carnea* extracts was evaluated for the hepatoprotective activity using antitubercular drugs induced liver toxicity in rat. Drug-induced liver toxicity is a potentially serious adverse effect of the currently used antitubercular chemotherapeutic regimens containing isoniazid, rifampicin and pyrazinamide. All these drugs are potentially hepatotoxic independently, when given in combination their toxic effects are enhanced in a synergistic manner. The conversion of monoacetyl hydrazine, a metabolite of isoniazid, to a toxic metabolite via cytochrome P_{450} leads to hepatotoxicity. Rifampicin induces cytochrome P_{450} enzyme causing an increased production of toxic metabolites from acetyl hydrazine (AcHz). Rifampicin can also increase the metabolism of isoniazid to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of isoniazid) is shortened by rifampicin and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by isoniazid and rifampicin in combination[29]. Pyrazinamide, in combination with isoniazid and rifampicin is also, associated with an increased incidence of hepatotoxicity[30]. In addition to these mechanisms, oxidative stress induced hepatic injury is one of the important mechanisms in hepatotoxicity produced by antitubercular drugs[31].

The present study revealed a significant increase in the level of AST, ALT, ALP and serum bilirubin levels of group II on exposure to antitubercular drugs, indicating considerable hepatocellular injury. Elevated levels of these enzymes in serum are presumptive markers of drug induced necrotic lesions in the hepatocytes[32]. Enhanced susceptibility of hepatocytes cell membrane to antitubercular drugs induced peroxidative damage might have resulted in increased release of these diagnostic marker enzyme levels into the systemic circulation[33]. The activity of ALT and AST are sensitive indicators of acute hepatic necrosis, and the ALP level is known to be indicative of hepatobilliary disease[34]. The results of the present study suggested that *I. carnea* extracts possess hepatoprotective activity against the hepatotoxicity induced by the combination of three antitubercular drugs. In our study the rise in liver serum markers levels induced by anti-tubercular drug administration was significantly reduced by *I. carnea* extracts (125, 250 and 500 mg/kg) pre-treatment suggesting that its hepatoprotective activity might be due to its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes. As an alternative to induce cellular damage by covalent binding, there is evidence that these antitubercular drugs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of hepatic antioxidant defence system. Many antibiotics therapy can favour free radical production and cause cellular damage[35]. The role of oxidative stress in the mechanism of antitubercular drugs induced hepatitis has been reported by Evan *et al*[36]. The body has an effective defence mechanism to prevent and neutralize the free radical induced damage. This is proficient by a set of endogenous antioxidant enzymes such as SOD, and CAT. These enzymes constitute a mutually supportive team of defence against reactive oxygen species[37]. The significantly reduced activities of SOD and CAT observed point out the hepatic damage in the rats after administered with antitubercular drugs but on treatment with 125, 250 and 500 mg/kg of *I. carnea* extracts groups showed significant increase in the level of these enzymes which indicates the antioxidant activity of the *I. carnea* extracts. The combination of antitubercular treatment in experimental animals enhanced lipid peroxidation, indicating increased oxidative stress in liver[38]. Thiobarbituric acid reactive substances are produced by products of LPO that occurs in hydrophobic core of bio-membranes[39]. Increase in the level of lipid peroxides in liver reflected the hepatocellular damage. The depletion of antioxidant defences and/or raise in free radical production deteriorates the prooxidant-antioxidant balance, leading to oxidative stress-induced cell death[40]. Depletion of reduced GSH is known to result in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased glutathione consumption as observed in the present study[41]. Post-treatment of the rats with *I. carnea* extracts significantly reduced the elevated levels of thiobarbituric acid reactive substances and increased the concentration of GSH and these results suggest that the hepatoprotective action of *I. carnea* extracts might be due to the presence of antioxidants like flavones and alkaloid[14]. From the results it is clear that *I. carnea* extracts have exhibited dose dependent activity, however the dose level of 500 mg/kg, and *p.o.* showed greater activity as compared to control and standard groups.

Pathological processes that interfere with the production of ATP may interfere with sodium pump activity, which in turn results in decreased hepatocellular function. It has been hypothesized that oxidative damage of membrane bound ATPase activity is crucial for mitochondrial membrane damage[42]. A significant depletion was found in the enzymatic activity of ATPase after antitubercular drug intoxication in experimental animals which was responsible for impaired function of the respiratory chain and ATP metabolism and the damage of the cellular membrane due to lipid peroxidation also led to

decrease in the activity of endoplasmic reticulum membrane bound enzyme such as G-6-Pase[43]. Our studies showed the acute exposure to antitubercular drug in rats significantly decreased ATPase and G-6-Pase activities in liver which might be due to the membrane fragility and permeability of the organs. Therapy of *I. carnea* extracts significantly restored the metabolic enzyme activities which indicated the improved the physiological functions in liver tissue.

The hepatoprotective effect of the *I. carnea* extracts was further accomplished by the histopathological examinations. *I. carnea* extracts at different dose levels offers hepatoprotection, but 500 mg/kg is more effective than all other lower doses. Liver histopathology images evidenced that *I. carnea* extracts attenuated the hepatocellular necrosis and led to reduction in inflammatory cells infiltration, may be attributed to its hepatoprotective effects. From the results it is clear that the *I. carnea* extracts has shown dose dependent activity among which the dose level of 500 mg/kg, *p.o.* shows greater activity which is comparable with the control and standard groups.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Drug-induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Therefore, present study was designed to demonstrate the anti-hepatotoxic and antioxidant potential of *I. carnea* against anti-tubercular drugs induced acute hepatopathy in experimental animals.

Research frontiers

The drug induced hepatotoxicity is a major concern in the recent therapeutic scenario. Mostly the drugs from natural origin are widely used to treat the hepatic disorder caused by biological and chemical agent, while screening of ethanolic extract of *I. carnea* shows significant hepatoprotective activity against selected antitubercular drugs *i.e.* rifampicin, isoniazid and pyrazinamide. Further study is required to investigate its hepatoprotective potential against drugs like aspirin, thioacetamide, carbon tetra chloride *etc*. induced liver toxicity.

Related reports

Sana and Javed (2010) stated that many antibiotics therapy can favour free radical production and cause cellular damage. And the role of oxidative stress in the mechanism of antitubercular drugs induced hepatitis has been reported by Evan *et al* (2010).

Innovations and breakthroughs

From the results it is clear that the *I. carnea* extracts has shown dose dependent activity among which the dose level of 500 mg/kg, *p.o.* shows greater activity which is comparable with the control and standard groups. Liver histopathology images clearly evidenced that *I. carnea* extracts attenuated the hepatocellular necrosis and led to reduction in inflammatory cells infiltration and attributed to its hepatoprotective effects.

Applications

The treatment with *I. carnea* extracts significantly prevent the drug induced increase in serum levels of hepatic enzymes and significantly reduced the lipid peroxidation in the liver tissue and restored activities of defence antioxidant enzymes GSH, SOD and CAT towards normal levels. Thus, *I. carnea* extracts is used for the management of hepatopathy and anti-oxidant.

Peer review

This is a unique work by which the authors evaluated anti-hepatotoxic and antioxidant influence of *I. carnea* against anti-tubercular drugs induced acute hepatopathy. The *I. carnea* extracts showed significant results as compared with the standard.

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