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A PRELIMINARY BIOCHEMICAL SCREENING OF *HELICTERES ISORA* L. STEM BARK IN CARBON TETRACHLORIDE INDUCED TOXICITY IN RATS

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Summary

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Helicteres isora, a tall shrub common in central and western India is an effective antidote to snake bite and is also used for various intestinal disorders. The potential efficacy for hepatocellular regeneration of the ethanolic extract of *Helicteres isora* L. at doses of 150 and 250 mg/kg was investigated in CCl_4-induced hepatic damage in rats for 21 days. Significant hepatoprotective effects were obtained against toxin induced liver damage as evident from assessed biochemical markers such as acid phosphatase (ACP), alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total protein, and oxidative stress parameters as thiobarbituric acid reactive substances (TBARS), catalase (CAT), glutathione (GSH) and glutathione-*S*-transferase (GST) in liver, kidney and plasma. The results revealed that at the dose of 250 mg/kg, *Helicteres isora* L. had a hepatocurative potential after CCl_4 intoxication in rats and provided a scientific rationale for the traditional use of this plant in the management of liver diseases.

Key words: antioxidant, carbon tetrachloride (CCl_4), *H. isora* L*,* hepatoprotection, rats

INTRODUCTION

More than 600 medicinal agents and chemical toxins have been reported to induce liver diseases (Lewis & Rawlins, 1995). They account for 20 % to 40 % of all instances of fulminant hepatic failure. The compounds, most commonly used in experiments on liver toxicity, are carbon tetrachloride, acetaminophen, cadmium chloride, ethanol and allyl chloride.

Carbon tetrachloride (CCl_4) induces successive hepatic damages consisting of hepatic steatosis, fibrosis, massive infiltration and cirrhosis **(**Schaff *et al.*, 1991**)**. It is widely used as an industrial solvent and for disinfestation of grain (Dikshith *et al.*, 1997). The CCl_4-induced hepatic damage is due to generation of free radicals, lipid peroxidation and the resulting decreased activities of antioxidant enzymes (Castro *et al.*, 1974; Poli, 1993).

Cleavage of carbon-chloride bonds results in the formation of trichloromethyl radicals which are highly unstable and react immediately with membrane components. They form covalent bonds with unsaturated fatty acids of membrane lipids resulting in

the production of chloroform and lipid peroxidation (Timbrell, 2000).

In recent years, the prevalence of fatty liver, cirrhosis and liver tumours in our country increased. Majority of these disorders have been linked to oxidative stress and free radicals. So, antioxidant therapy has gained an almost inevitable importance.

Plants are a rich source of natural antioxidants. A number of plants have been shown to possess hepatoprotective properties. Indian medicinal plants belonging to about 40 families have been investigated as liver protective drugs (Trivedi & Rawal, 2001). Many studies have been undertaken with traditional medicines, attempting to develop new drugs for hepatitis (Liu, 1989). The herbal medicines are comparatively safe and environment-friendly. They play an important role in health care programmes world wide and there is a resurgence of interest in herbal medicines for treatment of various hepatopathies (Venukumar & Latha, 2002).

Helicteres isora L. is a shrub or small tree available in forests throughout the central and western India. The roots and the bark are expectorant and demulcent. They are useful to treat colic, scabies, gastropathy, diabetes, diarrhoea, and dysentery (Kirtikar & Basu, 1995). The fruits are astringent, refrigerant, stomachic, vermifugal, vulnerary and are useful to control griping of bowels and flatulence of children (Chopra *et al.*, 1956), and have also antispasmodic effects (Pohocha & Grampurohit, 2001).

The aqueous extract of bark of *H. isora* plant is reported to have antioxidant activity (Kumar *et al.*, 2007; 2008). The LD_{50} value of *H.isora* bark is found to be greater than 2000 mg/kg in rats (Kumar *et al.*, 2007). Root extracts of *H. isora* possess antihyperglycaemic activity in glucose-loaded rats **(**Venkatesh *et al.*, 2004). It lowers plasma glucose and can nullify the insulin resistance activity (Chakrabarti *et al.,* 2002). A hypolipidaemic activity of *H. isora* has also been reported by Chakrabarti *et al.* (2002).

The present study has been undertaken to investigate the hepatocurative effects of *Helicteres isora* extract in CCl_4-induced hepatotoxicity in albino Wistar rats.

MATERIALS AND METHODS

Plant extract

The barks of *Helicteres isora* L. were collected from Kollimalai and Namakkal District of Tamilnadu, India. They were authentified at the Rabinot herbarium, Trichy and Botanical Survey of India, Coimbatore, Tamilnadu, shade dried and coarsely powdered. The extract of the same was made with ethanol using a Soxhlet apparatus. The extract was concentrated *in vaccuo*. The yield of extract was calculated as 6.23%. The brown coloured semi-solid extract was used in experiments.

Animals

Healthy Wistar albino rats (n=24) weighing 150–200 g were obtained from the SASTRA, Animal House, Thanjavur, Tamilnadu. They were maintained in controlled temperature (23 ± 3 °C) and humidity 60–65 % with 12 hrs dark and light cycle at CARISM Animal House, SASTRA, Thanjavur. The animals were fed commercial diet (Tetragon chemie pvt. Ltd., Doddaballapur, Bangalore). The study was approved by the Institutional Animal Ethical Committee with Reg No. 817/04/ac/cpcsea. The animals were divided into 4 groups of 6 rats each. Group I served as control, received the normal feed and was left untreated. Groups II, III and IV were treated intraperitoneally with

1 mL/kg 30 % CCl_4 in liquid paraffin thrice a week for 21 days. In addition to CCl_4, rats from groups III and IV received *H. isora* bark extract orally at daily doses of 150 and 250 mg/kg respectively, for 21 days.

Biochemical analysis

At the end of the experimental period, the animals were anaesthetized with pentobarbitone sodium at the dose of 65 mg/kg. Then they were killed by carotid cutting and blood was collected for separation of plasma. The liver and kidneys were dissected out immediately and transferred into ice-cold physiological saline for biochemical estimations. Tissue 10 % homogenates were prepared in 0.1M Tris HCl buffer.

The hepatoprotective effect of *H. isora* L. in CCl_4-intoxicated rats was assessed in tissue homogenates and plasma, by the biochemical markers aspartate (ASAT) and alanine (ALAT) aminotransferases (Reitman & Frankel, 1957), alkaline (ALP) and acid (ACP) phosphatases (King & Armstrong, 1934), and total protein (Lowry *et al.,* 1951). Oxidative stress markers as thiobarbituric acid reactive substances (TBARS) (Ohkawa *et al.,* 1979), reduced glutathione (Beutler *et al.,* 1963), glutathione-S-transferase (Habig *et al.,* 1974) and catalase (Aebi, 1983) were also assayed.

Statistical analysis

The results are presented as mean ± SEM. Statistical analysis was carried out using one-way ANOVA (DMRT test) and regression analysis (SPSS software v. 12.0.).

RESULTS

CCl_4 hepatotoxicity produced a significant elevation of ASAT level ($P<0.05$, Table 1) in plasma and liver homogenate. The severe hepatocellular injury was reduced after the *H. isora* treatment. The ASAT level in plasma and liver homogenate was reduced significantly at 250 mg/kg, showing the efficacy of our herbal extract ($P<0.05$ vs intoxicated rats).

Positive correlation was also observed between the TBARS and the liver marker ASAT ($r=0.727$) in plasma. These results reveal that the *H. isora* treatment can protect the liver from damage caused by lipid peroxides.

In our study the ALAT level was elevated significantly ($P<0.05$, Table 1) in liver and kidney homogenate of CCl_4-intoxicated rats, but was significantly lower ($P<0.05$ vs group II, Table 1) in the groups treated with *H. isora* extract. The levels gradually returned to normal in group IV, showing that the higher dose was more effective in toxicity.

A significant rise in the level of ALP in liver and kidney homogenate occurred in intoxicated untreated rats ($P<0.05$ vs control, Table 1). Administration of *H. isora* extract to CCl_4-treated rats significantly restored ($P<0.05$ vs the untreated intoxicated group) ALP activities at a dose of 250 mg/kg.

Similarly, ACP activity was significantly higher ($P<0.05$ vs controls, Table 1) in plasma and liver homogenate of CCl_4-intoxicated groups. After the treatment with *H. isora,* there was a significant reduction ($P<0.05$ vs group II) in ACP level when the higher dose was used (250 mg/kg).

The significant reduction in protein level in all samples of CCl_4-treated rats indicated the severity of liver disease ($P< 0.05$ vs controls, Table 1). Administration of *H. isora* to CCl_4-treated rats caused significant rise in protein concentrations at a dose of 250 mg/kg ($P<0.05$ vs group II).

Table 1. Liver biochemical markers in blood plasma, liver and kidney homogenates in rats, untreated (group I); treated i.p. with 1 mL/kg 30 % CCl_4 thrice for 21 days (group II) and CCl_4 –treated rats that received *H. isora* bark extract orally at daily doses of 150 mg/kg (group III) or 250 mg/kg (group IV) for 21 days. Data are presented as mean ± SEM; n=6

Groups	ALAT (IU/L)	ASAT (IU/L)	ALP (IU/L)	ACP (IU/L)	Total protein (g/dL)
Liver homogenate					
I (Control)	19±0.1	21±2.0	396±119.8	99±30.0	161±12.0
II (CCl_4)	60±4.6*	72±4.5*	837±4.8*	154±7.7*	128±7.7*
III (CCl_4 + *H. isora* 150 mg/kg)	47±3.6	50±2.4	652±111.2	123±7.6	156±18.6
IV (CCl_4 + *H. isora* 250 mg/kg)	30±6.1$^{\&}$	30±1.5$^{\&}$	450±20.7$^{\&}$	100±4.2$^{\&}$	252±42.7$^{\&}$
Blood plasma					
I (Control)	19±0.1	21±2.0	396±119.8	99±29.9	4±1.9
II (CCl_4)	17±2.2*	48±0.9*	1094±111.7*	204±9.0*	2±0.3*
III (CCl_4 + *H. isora* 150 mg/kg)	34±0.2$^{\&}$	23±2.1$^{\&}$	746±88.2	172±33.6	3±1.6$^{\&}$
IV (CCl_4 + *H. isora* 250 mg/kg)	22±2.2$^{\&}$	22±4.5$^{\&}$	429±119.7$^{\&}$	129±6.6$^{\&}$	3±1.5$^{\&}$
Kidney homogenate					
I (Control)	19±0.1	21±2.0	396±119.8	2476±749.0	448±79.6
II (CCl_4)	54±2.1*	20±8.3*	845±33.5*	1600±100.0*	214±19.3*
III (CCl_4 + *H. isora* 150 mg/kg)	22±3.2	49±0.9	1029±55.0	4327±228.0$^{\&}$	386±6.1
IV (CCl_4 + *H. isora* 250 mg/kg)	16±5.3$^{\&}$	50±0.7$^{\&}$	592±53.0$^{\&}$	2872±225.0$^{\&}$	562±57.8$^{\&}$

* $P<0.05$ vs group I (control); $^{\&}$ $P<0.05$ vs group II (CCl_4-treated).

In CCl_4-intoxicated rats, TBARS were statistically significantly elevated in plasma and kidney homogenate compared to controls (Table 2). In group IV, treated with *H. isora* at the higher dose (250 mg/kg), TBARS levels were significantly decreased in kidney homogenate ($P<0.05$, vs untreated intoxicated rats).

The findings in this study reveal that the activity of catalase was significantly lowered in CCl_4-intoxicated rats due to their susceptibility to oxidative injury ($P<0.05$ vs group I, Table 2). However, the overexpression of the antioxidant molecule after the administration of *H. isora* was indicative of its ability to activate hepatocellular antioxidant defense in the liver and the level was especially increased at a dose of 250 mg/kg ($P<0.05$ vs group II).

In our study, the GSH activity was reduced markedly in all kinds of samples obtained from intoxicated animals ($P< 0.05$ vs control) but it was enhanced by the treatment with *H. isora* at a dose of 250 mg/kg ($P<0.05$ vs the intoxicated group).

In CCl_4-intoxicated rats, the activity of GST was also significantly reduced in plasma, liver and kidney homogenate ($P<0.05$) compared to the control group. The administration of *H. isora* extract at 250 mg/kg significantly increased ($P<0.05$ vs group II, Table 2) the level of GST in

the respective samples showing that the activity of GST related to detoxication is increased towards normalization. This was also confirmed by the positive correlation observed between GST and GSH in liver samples (r=0.958).

DISCUSSION

ASAT is found in many tissues including liver, heart, skeletal muscle, kidney and brain. The release of large quantities of ASAT into the bloodstream is often associated with massive necrosis of the liver (Rees & Spector, 1961).

ALAT is found exclusively in the cytosol of the liver and kidney cells. The presence of ALAT in plasma is directly related to membrane damage. ALAT is a cytosolic enzyme, more specific for liver than ASAT. In acute inflammatory conditions of the liver, ALAT elevations are expected to be higher than those of ASAT and tend to be remain elevated. CCl_4 overdose causes a dramatic increase in ALAT activity (Ray *et al.*, 1996). Administration of CCl_4 also causes assimilation of fat in the liver leading to the increased ACP activity. This may be also due

Table 2. Oxidative stress markers and levels of antioxidants in blood plasma, liver and kidney homogenates in rats, untreated (group I); treated i.p. with 1 mL/kg 30 % CCl_4 thrice for 21 days (group II) and CCl_4 –treated rats that received *H.isora* bark orally extract at daily doses of 150 mg/kg (group III) or 250 mg/kg (group IV) for 21 days. Data are presented as mean ± SEM; n=6

Groups	TBARS (nmol MDA liberated/mg protein)	Catalase (μmol H_2O_2 used/min/mg of protein)	GSH (μg GSH/mg of protein)	GST (mg protein/mL of enzyme)
Liver homogenate				
I (Control)	0.48±0.08	162.50±22.96	6.12±0.15	8.42±0.28
II (CCl_4)	0.52±0.05*	43.30±9.30*	2.96±0.12*	4.14±0.16*
III (CCl_4 + *H. isora* 150 mg/kg)	0.48±0.49	82.63±11.33	5.99±0.13$^{\&}$	7.29±0.18
IV (CCl_4 + *H. isora* 250 mg/kg)	0.47±0.22$^{\&}$	120.52±7.17$^{\&}$	6.25±0.15$^{\&}$	8.02±0.21$^{\&}$
Blood plasma				
I (Control)	0.22±0.10	26.6±6.6	2.13±0.02	1.15±0.02
II (CCl_4)	1.30±0.02*	12.2±8.6*	1.38±0.01*	0.06±0.03*
III (CCl_4 + *H. isora* 150 mg/kg)	0.83±0.61	28.1±4.7$^{\&}$	1.88±0.02	1.78±0.04$^{\&}$
IV (CCl_4 + *H. isora* 250 mg/kg)	0.57±0.32$^{\&}$	29.5±5.9$^{\&}$	2.20±0.02$^{\&}$	2.10±0.03$^{\&}$
Kidney homogenate				
I (Control)	0.37±0.19	195.5±125.7	4.43±0.09	0.21±0.02
II (CCl_4)	1.60±0.10*	45.6±2.5*	2.07±0.09*	1.65±0.19*
III (CCl_4 + *H. isora* 150 mg/kg)	0.65±0.25	104.5±20.98	4.07±0.16	5.87±0.42$^{\&}$
IV (CCl_4 + *H. isora* 250 mg/kg)	0.30±0.21$^{\&}$	184.5±26.87$^{\&}$	5.15±0.39$^{\&}$	6.39±0.40$^{\&}$

* $P<0.05$ vs group I (control); & $P<0.05$ vs group II (CCl_4-treated).

to the lysosomal imbalance resulting in the destruction of the intact membranes (Abraham & Wilfred, 2000).

CCl_4 is bio-transformed by the cytochrome P450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($\bullet CCl_3$). Then, the trichloromethyl free radical combines with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical that attacks the membrane of endoplasmic reticulum, elicits lipid peroxidation, disrupts Ca^{2+} homeostasis and finally, results in cell death. These events result in altered structures of the endoplasmic reticulum and outer membranes and also, in reduction of protein synthesis (Recknagel & Glende, 1973; Gravela *et al.*, 1979; Wolf *et al.*, 1980; Azri *et al.*, 1992).

TBARS concentration is considered as a valuable indicator of drug- or toxicant-induced hepatic damage resulting from the oxidative stress. The level of lipid peroxidation was significantly increased (Table 2) in CCl_4-intoxicated groups, a clear manifestation of excessive formation of free radicals and activation of LPO system.

Catalase is one of the important enzymes in the supportive team of defense against reactive oxygen species (ROS). Catalase is a haemoprotein containing four haeme groups. that catalyses the decomposition of H_2O_2 to water and O_2 and thus, protects the cell from oxidative damage by H_2O_2 and $OH^{\cdot}$ (Tolbert, 1981).

GSH is a cellular non-protein sulfhydryl molecule that plays a vital role in protecting cells from oxidative damage. Furthermore, a role of glutathione in stabilizing biological membranes has been suggested (Costagliola *et al.,* 1985). A decrease in cellular GSH content increases oxidative stress since GSH serves as oxygen radical scavenger. Deficiency of cellular GSH increases prooxidant production and promotes cell death (Son *et al.*, 2001; Kim *et al.*, 2003).

Glutathione-S-transferase is another scavenging enzyme that binds to many different lipophilic compounds. GST catalyzes the conjugation of the thiol functional groups of glutathione to electrophilic xenobiotics and increases their solubility. Then the xenobiotic-GSH conjugate is either eliminated or converted to mercapturic acid. Since GST increases solubility of hydrophobic substances, it plays an important role in storage and excretion of xenobiotics. Compounds that increase the activity of GST are thought to have an increased hepatoprotective activity. The depression of hepatic GST activity might be an adaptive response to the increased production of oxidized glutathione in the tissue of CCl_4-hepatotoxic animals since the efflux of oxidized glutathione and GST use the same transport system. The moderate decline in GST activity in liver, seen in this study, might be due to the decreased availability of GSH (Boyer *et al.*, 1984; Masukawa & Iwata, 1986).

In conclusion, the evaluation of the hepatoprotective effect of *Helicteres isora in* CCl_4-intoxicated rats showed a total reversal and recovery of all biochemical and antioxidant markers studied following the drug administration. The treatment with *Helicteres isora* extract has shown a dose- and duration dependent activity, with a lesser effect at the lower dose (150 mg/kg) and a beneficial effect at the higher tested dose (250 mg/kg).

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