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Hepatoprotective activity of *Hemidesmus indicus* R. Br. in rats

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Treatment of rats with paracetamol and CCl_4 produced a significant increase in the levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total and direct bilirubin. Rats pretreated with methanolic extract of roots of *H. indicus* (100-500 mg/kg body weight, po) exhibited rise in the levels of these enzymes but it was significantly less as compared to those treated with paracetamol or CCl_4 alone. The results of methanolic extract of *H. indicus* were comparable with the standard hepatoprotective agent silymarin (100 mg/kg). Maximum hepatoprotective effect was found to be at the dose of 250 mg/kg body weight in case of CCl_4 induced hepatic damage while 500 mg/kg body weight in case of paracetamol induced hepatic damage. The results suggest that methanolic extract of *H. indicus* roots possesses a potential antihepatotoxic activity.

Keywords: CCl_4, *Hemidesmus indicus*, Hepatoprotective, Paracetamol.

Hemidesmus indicus R.Br. (Asclepiadaceae) known as *Indian Sarsaparilla* or *Anantmul*, is a well-known plant in Ayurvedic system of medicine. The plant is distributed throughout India in plains and low hills[1]. The root is sweet bitter, cooling, aphrodisiac, antipyretic and cures leprosy, leucoderma, asthma, bronchitis and general debility[2, 3]. Traditionally it is used as blood purifier, diuretic antirheumatic and antidote for snake bite[4]. Roots are reported to have antimicrobial[5] and anti-inflammatory[6,7] properties. Studies with methanolic extract of root bark of *H. indicus* have shown protection against rifampicin and isoniazide induced hepatic damage[8]. Root bark has been reported to possess antioxidant activity[9]. Some important chemical constituents of the root include hemidesmin I, hemidesmin II, amyrins, and lupeol 2-hydroxy 4-methoxy benzoic acid and some triterpenes[10-12]. From aerial parts of the plant, several pregnan steroids and glycosides have been isolated[13-16]. Coumarinolignoids found in *H. indicus* are important class of natural product that have shown enormous and potential biological activities including hepatoprotective and antioxidant activity[17]. Hemidesmin I, Hemidesmin II and flavonoid glycosides are major phytoconstituents found in *H. indicus*.

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Reactive oxygen species and free radicals play an important role in the etiology of various diseases such as inflammation, cataract, atherosclerosis, rheumatism, arthritis, ischemia reperfusion injury including liver disorders[18]. Paracetamol and CCl_4 share a common property of being converted into their respective reactive metabolites viz. N-acetyl p-benzoquinoneimine (NAPQI) and halogenated free radical by hepatic cytochrome P-450 (ref. 19). Therefore, in the present study, the hepatoprotective effect of methanolic extract of *H. indicus* has been evaluated in paracetamol and CCl_4 induced acute liver damage in the rats.

Materials and Methods

CCl_4 was procured from E. Merck (India) Ltd. Mumbai; silymarin was obtained as gift sample from Cadila Pharma Ltd., India. Paracetamol was obtained as gift sample from Torrent Research Center, Ahmedabad. Standard kit of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin was obtained from Span Diagnostics Ltd. All other reagents used for the experiments were of analytical grade.

Preparation of methanolic extract of root of H. indicus—The root of *H. indicus* was collected from wildly grown plant and authenticated in our Pharmacognosy Department by a Botanist and a voucher specimen (KB-PD 08/01) was preserved. It was air

dried and powdered to 40 mesh and stored in airtight container till further use. Powder (500 g) was defatted by petroleum ether and extracted with methanol using Soxhlet apparatus and the solvent was evaporated under reduced pressure. The methanolic extract was subjected to preliminary phytochemical testing for detection of major chemical groups[20].

Animals—Wistar albino rats of either sex weighing between 150-200 g were used for the hepatoprotective study. The animals were housed in polypropylene cages and maintained at 24°±2°C under 12 hr light/dark cycle and were fed *ad libitum* with standard pellet diet and had free access to water. They were initially acclimatized for the study and the study protocol was approved by the Institutional Animal Ethics Committee as per the requirements of Committee for the Purpose of Control and Supervision on Animals (CPCSEA), New Delhi.

Experimental protocol for hepatoprotective study—(a) CCl_4 induced hepatotoxicity: Rats were divided into 5 groups of 5 animals each. Group I served as vehicle control and received normal saline (5 ml/kg). Group II was administered with CCl_4/olive oil (1:1, v/v, 0.7 ml/kg, ip on alternate days)[21, 22]. Animals in Group III and IV and V received methanolic extract (100, 250 and 500 mg/kg, po respectively daily for 7 days) simultaneously with toxicant (CCl_4/olive) oil. Group VI was administered with reference drug, silymarin[23] (100 mg/kg, po) simultaneously with toxicant.

(b) *Paracetamol induced hepatotoxicity*—In the paracetamol induced liver injury model, paracetamol (2 g/kg po) suspension prepared using carboxy methyl cellulose was administered to all animals except the animals of the normal control group on 6th day. Silymarin (100 mg/kg po) was used as a standard. Group I, which served as normal control, received distilled water, ip. Group II received paracetamol (2 g/kg, po) single dose on 6th day. Group III received paracetamol (2 g/kg, po) single dose and silymarin (100 mg/kg, po) simultaneously for 7 days. Group IV received paracetamol 2 g/kg po single dose and methanolic extract (100 mg/kg po) simultaneously for same period. Group V received paracetamol (2 g/kg po) single dose and methanolic extract (250 mg/kg po) simultaneously for same period. Group VI received paracetamol (2 g/kg, po) single dose and methanolic extract (500 mg/kg, po) simultaneously for 7 days.

Assessment of hepatoprotective activity—On the seventh day of the start of respective treatment the rats were anaesthetized by light ether anaesthesia and the blood was withdrawn by making intracardiac puncture to the rats. It was allowed to coagulate for 30 min and serum was separated by centrifugation at 2500 rpm. The serum was used to estimate serum glutamate pyruvate transaminase (SGPT) [24] serum glutamate oxaloacetate transaminase (SGOT)[24] alkaline phosphatase (ALP)[25] total bilirubin[26] and direct bilirubin.

The results of antihepatotoxic activity were presented as the mean±SE of 5 animals each group. Results were analyzed statistically using analysis of variance (ANOVA) followed by Tukeys test. Values of $P<0.05$ were considered significant.

Results

Preliminary phytochemical screening indicated the presence of tannin, flavonoids, saponins and sugars. There was significant elevation of SGOT, SGPT, ALP and bilirubin (total and direct) levels in the CCl_4 treated groups (Table 1). In groups orally treated with 100, 250 and 500 mg/kg of aqueous suspensions of methanolic extract of *H. indicus* and silymarin (100 mg/kg), above activities of enzymes were found to be significantly ($P<0.05$) decreased as compared to the CCl_4 treated group. Maximum protection by methanolic extract of *H. indicus* against CCl_4 induced hepatic damage was offered at the dose of 250 mg/kg (Table1).

Like CCl_4, paracetamol treated animals showed elevation of SGOT, SGPT, ALP and Bilirubin (total and direct) levels as compared to vehicle treated normal control group. In groups orally treated with 100 and 250 mg/kg of aqueous suspension of methanolic extract and silymarin, above activities of enzymes were found to be significantly decreased ($P<0.05$) as compared to the paracetamol treated control group. In case of paracetamol induced hepatic damage maximum protection was offered at the dose of 500 mg/kg body weight (Table 1).

Discussion

Paracetamol and CCl_4 induced hepatic injuries are commonly used models for the screening of hepatoprotective drugs[28,29] and the extent of hepatic damage is assessed by the level of released cytoplasmic alkaline phosphatase and transaminases (GOT and GPT) in circulation[30]. The present investigation also revealed that the given dose of CCl_4 (0.7 ml/kg, ip) and paracetamol (2 g/kg) produced significant elevation in SGPT, SGOT and alkaline phosphatase levels indicating an impaired liver function. The massive production

Table 1— Effect of methanolic extract of *H. indicus* on CCl_4 and paracetamol induced hepatotoxicity
[Values are mean ±SE from 5 animals in each group]

Group	Serum biochemical parameters				
	SGPT (U/ml)	SGOT (U/ml)	Alkaline phosphatase (K.A.Units)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
NormalControl	31.70 ± 4.56	38.95±7.51	12.99±0.45	0.87±0.04	0.16±0.09
CCl_4 (0.7 ml/kg)	206.62±7.06[a]	197.58±6.10[a]	39.82±1.54[a]	2.59±0.42[a]	0.851±0.20[a]
MeOH ext 100 mg/kg + CCl_4	89.37±4.24[b]	101.89±4.0[b]	27.13±0.08[b]	1.58±0.38[b]	0.29± 0.08[b]
MeOH ext 250 mg/kg + CCl_4	46.04±2.27[c]	76.63±7.74[c]	18.66±0.42[c]	1.18±0.2[c]	0.26±0.09[c]
MeOH ext 500 mg/kg + CCl_4	57.30±3.2[c]	85.43±6.32[c]	17.39±0.92[c]	1.43±0.29[c]	0.29±0.018[c]
Silymarin 100 mg/kg + CCl_4	44.95±5.51[c]	87.65±5.03[c]	16.25±0.15[c]	0.96±0.13[c]	0.25±0.09[c]
Paracetamol 2 gm/kg	254.38±17.13[a]	564.64± 37.73[a]	45.58 ±0.43[a]	2.78±0.55[a]	0.92 ± 0.15[a]
MeOH ext 100 mg/kg + paracetamol	69.45±3.64[d]	110.15±2.62[d]	23.67±0.55[e]	1.49±0.13[d]	0.64±0.09[d]
MeOH ext 250 mg/kg + paracetamol	59.04± 3.40[e]	96.65±4.83[e]	20.18±0.37[e]	1.33±0.19[e]	0.60±0.01[e]
MeOH ext 500 mg/kg + paracetamol	48.92±4.01[e]	77.59±4.23[e]	18.88±0.72[e]	1.29±0.1[e]	0.72±0.1[e]
Silymarin 100 mg/kg + paracetamol	16.19±1.71[e]	48.31±2.96[e]	14.01±0.15[e]	0.99±0.1[e]	0.33±0.02[e]

P values: [a] <0.001 when compared with normal control group, [b]<0.05 when compared with CCl_4 treated group, [c]<0.001 when compared with CCl_4 treated group, [d]<0.05 when compared with paracetamol treated group, [e]<0.001 when compared with paracetamol treated group.

of reactive species may lead to depletion of protective physiological moieties (glutathione and tocopherols etc.) and ensuing widespread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes. The investigation further reveals that the methanolic extract of *H. indicus* had been effective in offering protection, which is comparable to silymarin. The methanolic extract of *H. indicus* roots when administered to the rats exhibited protection against both paracetamol and CCl_4 induced liver injuries as manifested by the reduction in toxin mediated rise in serum enzymes. Both paracetamol and CCl_4 share a common property of being converted into their respective reactive metabolites N-acetyl p-benzoquinoneimine and halogenated free radical by hepatic cytochrome P-450 (ref. 19).

Paracetamol, an analgesic and antipyretic agent is safe in recommended doses but produces hepatic necrosis when ingested in very large doses. It is established that at these relatively large doses paracetamol is biotransformed into a reactive metabolite N-acetyl p-benzoquinoneimine (NAPQI) by cytochrome P-450 mixed function oxidase[31]. Paracetamol toxicity is enhanced by factors that cause GSH depletion, enhanced NAPQI formation, or reduction in the antioxidative capacity of the liver, it could be suggested that the partial hepatoprotection afforded by the methanolic extract may be ascribed to one or more of these factors.

Phytochemical investigations revealed that roots of *H. indicus* showed presence of flavonoids, coumarinolignans and tannins. The literature has already documented the antioxidant and hepatoprotective value of flavonoid and coumarinolignans[33]. Thus, it appears that the hepatoprotection offered by *H. indicus* extract may be related to its free radical scavenging activity.

It is thus concluded that methanolic extract of *H. indicus* roots exhibited antihepatotoxic effect against paracetamol and CCl_4 induced hepatic damage. Further studies in progress for isolation and characterization of phytoconstituents may lead to development of lead nucleus for hepatic dysfunction.

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