

Antioxidant and Antihepatotoxic Activities of *Hemidesmus indicus* R. Br

G.M. Mohana Rao^{1*}, Ch. Venkateswararao¹, A.K.S. Rawat, P. Pushpangadan¹ and A. Shirwaikar²

¹Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow- 226 001, Uttarpradesh, India.

²Department of Pharmacognosy, College of Pharmaceutical Sciences. Manipal- 576 119, Karnataka India.

Abstract

The Antioxidant and antihepatotoxic activities of 50% aqueous ethanolic extract of *Hemidesmus indicus* R. Br. roots were assessed in experimental animals. In carbontetrachloride induced hepatic challenge in rats, a remarkable elevation of lipid peroxidation and a decrease in liver antioxidant enzymes levels were observed. Pretreatment with the 50% aqueous ethanol extract of *Hemidesmus indicus* R. Br. roots inhibited these alterations. Based on these results we can suggest that in the liver protective and antioxidative effects of *H. indicus*, possibly involve mechanisms related to free radical scavenging effects.

Keywords: *Hemidesmus indicus*; Antioxidant activity; Antihepatotoxic activity, Free radicals.

Introduction

In spite of tremendous strides in the modern medicine Liver diseases remain one of the most serious health problems. Liver is an organ of paramount importance as it plays an essential role in maintaining the biological equilibrium of vertebrates. The reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$) have been implicated in the pathophysiology of various clinical disorders, including ischemia, reperfusion injury, atherosclerosis, acute hypertension, haemorrhagic shock, diabetes mellitus and cancer (Hemnani, and Parihar, 1998). They play an important role in the inflammation process after intoxication with by ethanol, carbon tetrachloride or carrageenan (Yoshikawa, *et al.*, 1983; Halliwell, and Gutteridge, 1984; Yuda, *et al.*, 1991). It is well known thing that these free radicals and the reactive species derived from them cause damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury (Brattin, *et al.*, 1985). Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals (Osawa, *et al.*, 1990). Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices as well as in traditional systems of medicine in India (Subramaniam, *et al.*, 1998). *Hemidesmus indicus* R. Br

* Corresponding author: e-mail: mmraos@rediffmail.com

(Asclepiadaceae), commonly known as 'Anantamul', is a well-known drug in Ayurvedic system of medicine. The root has anti-microbial properties (Satyavati, *et al.*, 1987) and potent anti-inflammatory activity (Dutta, *et al.*, 1982; Alam, *et al.*, 1998). 50% aqueous ethanolic extract of the root was found to be antihepatotoxic (Prabhakaran and Gomes, 2000). Some important chemical constituents of the root include hemidesmin I, hemidesmin II, α -amyrin, β -amyrin, lupeol, 2-hydroxy-4methoxy-benzoic acid and some triterpenes (Satyavati *et al.*, 1987; Das *et al.*, 1992; Alam *et al.*, 1994; Roy *et al.*, 2001). The present study was aimed to evaluate the relationship between liver protective effects and antioxidant activity of 50% aqueous ethanol extract of *H. indicus* roots.

Materials and Methods

Plant material

H. indicus roots were collected from coastal areas of Ongole, Andhrapradesh during April-May 2004, and authenticated by Dr. Vivek Kumar, National Botanical Research Institute, Lucknow, India. A voucher specimen was deposited in the herbarium (NBRs No-35/03).

Preparation of the extract

The coarsely powdered roots were Soxhlet extracted with 50% aqueous ethanol (1:19 w / v) for 8 h (9.5%). The extract was resuspended in water and used for preliminary antioxidant studies. Phytochemical screening (Wagner *et al.*, 1984; Harborne, 1973; Stahl, 1969) gave positive tests for flavonoids and terpenes.

Test Animals

Male Wistar rats (150-200g) purchased from CDRI, Lucknow were used. The animals were maintained under standardized environmental conditions (22-28°C, 65-70% relative humidity, 12-h dark/light cycle) and fed with standard rat feed (Amruth India Ltd) and water *ad libitum*.

Inhibition of superoxide radical production

The effect on the superoxide radical production was evaluated using nitro blue tetrazolium (NBT) reduction method (McCord *et al.*, 1969). The reaction mixture contained: EDTA (6 μ M; with 3 μ g NaCN), riboflavin (2 μ M), NBT (50 μ M), *H. indicus* extract (from 10 to 1000 μ g/ml) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent lamp for 15 min, and the optical density was measured at 530 nm before and after illumination.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging was measured by studying the competition between deoxyribose and the test compounds for hydroxy radicals generated from the Fe^{3+} / ascorbate / EDTA / H_2O_2 system (Elizabeth *et al.*, 1990). The reaction mixture contained: deoxyribose (2.8 mM), FeCl_3 (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM), KH_2PO_4 - KOH buffer (20 mM, pH 7.4), and the extract (from 10 to 3000 μ g/ml) in a final volume of 1 ml. After incubation for 1 h at 37°C, the deoxyribose degradation was measured as TBARS (Ohkawa *et al.*, 1979).

Nitric oxide radical inhibition activity

Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions, which are measured by Gries reaction (Green *et al.*, 1982; Marcocci *et al.*, 1994). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and the extract (from 10 to 1000 μ g / ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture was removed and 0.5 ml of Gries reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthaylene diamine

dihydrochloride) was added. The absorbance of the chromophore formed was evaluated at 546 nm.

Inhibition of Lipid Peroxide Formation

Induction by Fe²⁺ / ascorbate system

The reaction mixture containing rat liver homogenate (0.1 ml, 25% w / v) in Tris- HCl (30mM), ferrous ammonium sulfate (0.16 mM), ascorbic acid (0.6 mM), and different concentrations of the extract (from 10 to 1000 µg / ml) in a final volume of 0.5 ml was incubated for 1 h at 37°C (Biyashee and Balasubramanian, 1971) and the resulting thiobarbituric reacting substance (TBARS) was measured by method of Ohkawa *et al.*, 1979. A 0.4-ml aliquot of the reaction mixture was treated with sodium dodecyl sulfate (0.2 ml, 8.1%), thiobarbituric acid (1.5 ml, 0.8%), and acetic acid (1.5 ml, 20%, pH 3.5), made to a total volume of 4 ml by adding distilled water, and kept in a water bath at 95°C for 1 h. After cooling, distilled water (1 ml) and 5 ml of *n*-BuOH / py 15:1 (v/v) were added. After shaking and centrifugation, the organic layer was separated and the absorbance measured at 532 nm.

Induction by Fe³⁺ / ADP / ascorbate system

The reaction mixture (Sujioka *et al.*, 1987), containing rat liver homogenate (0.5 ml, 10% w/v), ferric chloride (100 µM), ADP (1.7 mM), ascorbic acid (500 µM), the extract (from 10 to 1000 µg/ml) and sufficient KCl (0.15 M) for final volume 1.5 ml, was incubated for 20 min at 37°C. Lipid peroxidation was determined by the thiobarbituric acid reaction according to Ohkawa *et al.*, 1979.

Hepatoprotective effect against acute dose of CCl₄ in rats.

Animals were divided into five groups of six rats each. Group 1 and 2, serving as normal and intoxicated control respectively, received only the vehicle (2% gum acacia, p.o.); group 3 was treated with quercetin (100 mg/kg, p.o. for 6 days) as standard; group 4 and 5 received the extract (200 and 400 mg/kg, p.o. respectively for 6 days). On day 4, 2 h after treatment, groups 2 – 5 rats received CCl₄ (0.25 ml / kg, i.p.) in liquid paraffin (1:1) (Nishigaki *et al.*, 1992). The animals were killed 48 h after the acute dose of CCl₄. The blood was collected by heart puncture and serum was separated by centrifugation (3000 rpm at 4°C for 10 min). The livers were immediately removed. Tissue and serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activity (Kind *et al.*, 1980), alkaline phosphate (ALP) activity (Reitman and Frankel, 1957) were measured.

Statistical analysis

Data were analyzed by Student's *t*-test. Differences below the 0.05 level ($P < 0.05$) were considered as significant.

Results and Discussion

The 50% aqueous ethanol extract of *Hemidesmus indicus* root was found to scavenge the superoxides generated by riboflavin photoreduction method with an IC₅₀ of 560 µg / ml while quercetin showed IC₅₀ of 7.5 µg/ml (Table 1). *H. indicus* inhibited hydroxyl radicals generated by Fe³⁺ / ascorbate / EDTA / H₂O₂ system with IC₅₀ of 2600 µg / ml in comparison with 30 µg / ml of quercetin (Table 1). Nitric oxide radical generation at physiological pH from sodium nitroprusside was found to be inhibited by *H. indicus* with an IC₅₀ of 660 µg / ml (Table 1). The extract was found to inhibit lipid peroxides generated by the induction of Fe²⁺ / ascorbate and Fe³⁺ / ADP / ascorbate in rat liver homogenates, IC₅₀ values being 610 and 990 µg / ml, respectively (Table 1).

A significant elevation was observed in serum and tissue ALP, GPT and GOT activities following CCl₄ intoxication in the rats. In the groups orally treated with 200 and 400 mg / kg of the extract, the above enzyme activities were found to decrease when compared to CCl₄ treated control group (Table 2 and Table 3).

On the basis of our results, it is revealed that *H. indicus* root extract possess free radical scavenging activity which could exert a beneficial action against liver damage induced by CCl₄. Since the preliminary phytochemical analysis of the extract shows the known presence of triterpenes and flavonoids (Satyavati *et al.*, 1987; Das *et al.*, 1992; Alam *et al.*, 1994; Roy *et al.*, 2001), we can suggest that these constituents may be responsible for the observed effects. In conclusion, the 50% aqueous ethanol extract of *H. indicus* exhibited a liver protective effect against CCl₄ induced hepatotoxicity and possessed anti-lipid peroxidative and free radical scavenging activities.

Table 1. Effect of the 50% aqueous ethanol extract of *H. indicus* on oxygen derived free radical generation invitro.

Test material	IC ₅₀ (µg/ml)				Nitric oxide
	Superoxide	Hydroxyl	Lipid peroxide		
			A	B	
<i>H. indicus</i>	560 ± 26	2600 ± 77	660 ± 44	610 ± 56	990 ± 68
Quercetin	7.5 ± 1.3	30 ± 6.7	8.5 ± 3	NM	NM

A, lipid peroxidation in Fe²⁺ / ascorbate; B, lipid peroxidation in Fe³⁺ / ADP / ascorbate; NM, not measured, Values are mean ± S.E.M. (n = 6).

Table 2. Effects of the 50% aqueous ethanol extract of *H. indicus* roots (p.o for 6 days) on tissue enzymatic changes in CCl₄ induced hepatotoxicity in rats.

Treatment	Dose (mg / kg, p.o.)	GOT (U / l)	GPT (U / l)	ALP (U / l)
Normal (vehicle)	-	118.0 ± 13.6*	89.0 ± 8.9*	183.0 ± 15.9*
Control		625 ± 28.0	845 ± 39.4	1028 ± 44.1*
Quercetin	100	93 ± 12.9*	123.8 ± 14.0*	283.9 ± 19.5*
<i>H. indicus</i>	200	443 ± 33.8*	240.1 ± 29.8*	483.0 ± 17.9*
	400	373 ± 18.9*	189.8 ± 24.8*	391.0 ± 26.9*

Values are mean ± S.D (n = 6); *P < 0.001 vs. control, Student's *t*-test. CCl₄ (0.25 ml / kg, i.p.) was administered to all groups except normal group on day 4.

Table 3. Effect of the 50% aqueous ethanol extract of *H. indicus* roots (p.o for 6 days) on serum enzymatic changes in CCl₄ induced hepatotoxicity in rats.

Treatment	Dose (mg / kg, p.o.)	SGOT (U / l)	SGPT (U / l)	ALP (U / l)
Normal (vehicle)	-	58.4 ± 7.2*	56.6 ± 5.2*	131.1 ± 16.1*
Control		187.8 ± 24.6	247.2 ± 38.1	234.8 ± 18.8
Quercetin	100	76.6 ± 9.2*	51.0 ± 3.1*	119.8 ± 7.8 *
<i>H. indicus</i>	200	212.2 ± 30.1	244.2 ± 35.0*	230.8 ± 21.2
	400	82.2 ± 5.18*	52.1 ± 7.1*	130.1 ± 20.1*

Values are mean ± S.D (*n* = 6); **P* < 0.001 vs. control, Student's *t*-test. CCl₄ (0.25 ml / kg, i.p.) was administered to all groups except normal group on day 4.

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