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Antioxidant potentials of *Hypericum hookerianum* (Family: Hypericaceae) on CCl_4 induced hepatotoxicity in rats

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SUMMARY

Free radicals are known to play important role in pathophysiology of hepatic disorders and antioxidants are employed along with other chemotherapeutic agents in treatment of such diseases. In search of natural antioxidant, successive extracts of *Hypericum (H.) hookerianum* (Family: Hypericaceae) were evaluated by *in vitro* and *in vivo* methods. Extracts of aerial parts of *H. hookerianum* were subjected for 1,1-diphenyl 2-picryl hydrazyl radical scavenging activity (DPPH assay), nitric oxide radicals scavenging assay and thiobarbituric acid reactive substances (TBARS) assay. Methanolic extract was found to be more active than other extracts in DPPH and *in vitro* TBARS assay with IC_{50} at 5.82 ± 1.33 μg/ml and 49.78 ± 3.79 μg/ml respectively. While petroleum ether extract showed more potentials in scavenging the nitric oxide radicals with IC_{50} 220.97 ± 2.69 μg/ml. The administration of CCl_4 to the control animals caused decrease in the level of catalase and superoxide dismutase, together with significant increase in the level of TBARS in liver and kidney. Reversal of these changes towards normal group was observed by administration of *H. hookerianum* methanolic extract at 50 and 100 mg/kg body weight, while other extracts were found to be less active.

Key words: *Hypericum hookerianum*; Antioxidant; Hepatoprotective; DPPH assay, NO scavenging, TBARS assay

INTRODUCTION

Free radicals so much so reactive oxygen species (ROS) play important role in the etiology of number of diseases including cardiovascular, ischemic disease, and aging processes (Halliwell *et al.*, 1992; Gutteridge, 1993; Halliwell and Gutteridge, 1995). When antioxidants are employed in the treatment of such diseases, they participate in body defense mechanism against ROS, which include variety of enzymes such as superoxide dismutase (SOD), which produces hydrogen peroxide from superoxide radicals, catalase (CAT) and glutathione-related enzymes, which decompose hydrogen peroxide (Halliwell, 1990; Trocino *et al.*, 1995). Excess production of free radicals impacts the pathogenesis and progression of various diseases (Visioli *et al.*, 2000). Lipid peroxides, produced from unsaturated fatty acids via free radicals causes toxic effects and promote the formation of additional free radicals in a chain reaction. Deficient activity of enzymes or scavengers which takes part in neutralizing these free radicals, leads to development of oxidative stress related diseases. The rationale for the use of antioxidants is well established in prevention and

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treatment of chronic diseases where oxidative stress plays a major role (Mukherjee, 2003; Mukherjee and Verpoorte, 2003; Rajlakshmi *et al.*, 2003). There are number of lipophilic and hydrophilic low-molecular-weight antioxidants, which directly reacts and scavenge the ROS (Sharma and Buetner, 1993).

Numbers of species belonging to genus *Hypericum (H.)* have been reported to possess varied bioactive potentials including antioxidant and hepatoprotective potentials. Different *H.* species had been studied and found to be potent antioxidant and hepatoprotective. Methanolic extract of *H. triquetrifolium* is reported to show significant antioxidant potentials (Conforti *et al.*, 2002). EI-Sherbiny *et al.* (2003) demonstrated antioxidant potentials of *H. perforatum* extract against elevated rat brain oxidative status, induced by amnestic dose of scopolamine. Antioxidant phenolic compounds (I3, II8-biapigenin, quercetin, quercetin-3-O-l-arabinofuranoside, quercetin-3-O-d-galactopyranoside, quercetin-3-O-d-galactopyranoside-7-O-dglucopyranoside) were isolated from *H. hyssopifolium* (Cakir *et al.*, 2003). Phytoconstituents such as proanthocyanidin and quercetin are also reported for their presence in *H. perforatum* and for their antioxidant potentials (Upton, 1997). Around 400 different species of *H.* are spread world wide, out of which about 20 are reported in India. *H. hokkerianum* Wight and Arn. is generally cultivated in gardens as ornamental plant in hilly regions (Anonymous, 1997). The plant has been reported for wound healing (Mukherjee and Suresh, 2000), antimicrobial (Mukherjee *et al.*, 2001a, 2003) and CNS active potentials (Mukherjee *et al.*, 2001b). Considering antioxidant and hepatoprotective potentials of other *H.* species, present studies were under taken to evaluate the antioxidant and hepatoprotective potentials of *H. hookerianum.*

MATERIALS AND METHODS

Plant materials and extraction

The fresh plant materials (aerial parts) of *H. hookerianum* were collected from different places of Nilgiris district of Tamil Nadu, India. Botanical Survey of India, Coimbatore, India, identified the collected plant material. Voucher specimen was preserved at our laboratory for the future reference. The plant material was dried under shade and pulverized by mechanical grinder, passed through 40 mesh sieve and stored in a closed container for future use. The powdered plant material was extracted successively with petroleum ether, chloroform, ethyl acetate and methanol in soxhlet apparatus. The material was extracted for 18 h with each solvent. The extracts were concentrated in rotary vacuum evaporator and dried under vacuum. Percentage yields of extracts were found to be 4.7%, 3.1%, 6.2% and 8.7% (w/w) with petroleum ether, chloroform, ethyl acetate and methanol respectively. The extracts were stored in well packed containers at 2 - 8°C, and subjected further for antioxidant evaluation.

Animals

Adult Wistar rats of both sexes (180 - 220 g) and Swiss albino mice (18 - 22 g), maintained under standard laboratory conditions with temperature of 25 ± 2°C and relative humidity of 30 - 70% were used. A 12 : 12/light:dark cycle was followed. Animals had free access to water and standard pellet laboratory animal diet (Hindustan Lever Ltd., India). All the experimental procedures and protocols used in this study were reviewed and approved by the institutional animal care and use committee and were in accordance with the guidelines of the committee for the purpose of control and supervision on experiments on animals (CPCSEA), Ministry of social justice and empowerment, Government of India.

Chemicals used

Petroleum ether, chloroform, ethyl acetate, methanol of analytical grade (E. Merck, Mumbai, India) were used in the experiment. 1,1-Diphenyl 2-picryl hydrazyl (DPPH) was obtained from Sigma Chemicals Co., (St. Louis, U.S.A.). Sodium nitroprusside, naphthyl

ethylene diamine dihydroch1oride, sulfanilic acid, glacial acetic acid, dimethyl sulfoxide (DMSO), sodium chloride, ascorbic acid, ferric chloride, sodium dodecyl sulphate, thiobarbaturic acid, n-butanol, pyridine, carboxyl methyl cellulose and other chemicals for the biochemical estimation were obtained from Rankem laboratory, Qualigen and SD Fine Chemicals (Mumbai). Spectrophotometer (Aquaris - CE 7200, CECIL Instruments Ltd, U.K.) was used to take absorbance of various reaction mixtures of the *in vitro* and *in vivo* studies.

1,1-Diphenyl-2-picryl hydrazyl radical scavenging activity (DPPH assay)

The antioxidant activity of the plant extracts and the standards were assessed on the basis of the radical scavenging effect of the DPPH free radical (Gamez *et al.*, 1998; Raja *et al.*, 2005a; Sundararajan *et al.*, 2006). The reaction mixtures contain 75 µl of DPPH solution (1.3 mg/ml), 100 µl of extract (test) solution and 2,825 µl of methanol. 3 different concentrations (10, 100 and 1,000 µg/ml) of test sample were used in the experiment. All the reaction mixtures were incubated for 15 min at 25°C. Gallic acid (0.5, 1, 2 and 4 µg/ml) was used as a standard for the comparison of the scavenging potentials (Hwang *et al.*, 2001; Mukherjee, 2002). The absorbance of each solution was determined at 517 nm using spectrophotometer (Aquaris - CE 7200, CECIL Instruments Ltd, U.K.). Unscavenged free radicals were estimated with respect to the corresponding blank readings. IC_{50} value, the concentration of the sample required to scavenge 50% DPPH free radical was determined through plotting the inhibition curve.

Nitric oxide radical inhibition assay

Nitric oxide radicals are generated by sodium nitroprusside in aqueous solution at physiological pH, on interaction with oxygen it produce nitrite ions, which can be estimated by the use of Griess Illosvoy reaction (Garrat, 1964). In this investigation, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Maracocci *et al.*, 1994; Mukherjee, 2002; Badami *et al.*, 2003). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and extract or standard (0.5 ml) was incubated at 25°C for 150 min. 0.5 ml of the reaction mixture containing nitrite was pipetted out and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization, followed by addition of 1 ml of naphthyl ethylene diamine dihydrochloride. The mixture was mixed well and allowed to stand for 30 min at 25°C. The absorbance of the solutions was measured at 540 nm against the corresponding blank solutions using spectrophotometer (Aquaris - CE 7200, CECIL Instruments Ltd, U.K.). 3 different concentrations (10, 100 and 1000 µg/ml in DMSO) of test sample were tested. In control solution vehicle (DMSO) was used instead of extract solution. Quercetin (12.5, 25, 50, 100 and 200 µg /ml) was used as standard to compare the scavenging potentials.

Lipid peroxidation assay

Rat liver homogenate preparation: Anaesthetized adult Wistar rats were dissected to take out the liver. The liver lobes were washed with 0.9% sodium chloride solution. Liver lobes were subjected for homogenization using Teflon homogenizer, in ratio 1 : 10 of 0.05 M phosphate buffer (pH 7.4). The homogenate was used for the estimation of thiobarbituric acid reactive substances (TBARS).

TBARS assay

The reaction mixture contained rat liver homogenate (0.25 ml), 0.1 ml 40 mM Tris-HCl buffer (pH 7.0), 0.05 ml 0.1 mM ascorbic acid, 0.05 ml 4 mM ferric chloride solution and 0.05 ml of test extract. 3 different concentrations (10, 100 and 1,000 µg/ml) of extracts were tested. Quercetin (12.5, 25, 50, 100 and 200 µg/ml) was used as a standard. The

mixtures were incubated at 37°C for 1 h, it was mixed with 0.2 ml sodium dodecyl sulphate (8.1%), 1.5 ml thiobarbituric acid (0.8%) and 1.5 ml acetic acid (20%). Volume was made up to 4 ml with distilled water. The mixtures were kept at 100°C in water bath for 1 h. The mixtures were cooled and 1 ml distilled water, 5 ml n-butanol and pyridine (15 : 1) were added. Mixtures were centrifuged at 4,000 rpm for 10 min and absorbance of organic layer was measured at 532 nm (Okhawa *et al.*, 1979; Raja *et al.*, 2005a; Sundararajan *et al.*, 2006).

Acute toxicity study

Swiss albino mice were divided in to test and control groups comprising of 6 animals in each group. The test was performed using increasing oral doses of successive extracts of *H. hookerianum* to each test group of the animals, in 10 ml/kg volume. Another group of mice was administered 0.3% CMC (10 ml/kg, p.o.) as control. The mice were allowed feed *ad libitum* during a 24 h test and kept under regular observation for mortality and behavioral changes.

***In vivo* antioxidant and hepatoprotective activity**

Wistar rats were divided into eleven groups comprising of six animals in each group. Group I served as normal and received 1 ml of 0.3% sodium CMC. Group II served as CCl_4 treated control and received 1 ml of 0.3% sodium CMC. Group III-IV and V-VI animals received the petroleum ether extract of *H. hookerianum* (HHPE) and chloroform extract of *H. hookerianum* (HHCL) respectively. Group VII-VIII and IX-X animals received the ethyl acetate extract of *H. hookerianum* (HHEA) and methanol extract of *H. hookerianum* (HHME) respectively. All the extracts were administered at dose of 50 and 100 mg/kg body weight for the convenience of the comparison and on the basis of toxicity studies. Group XI animals received standard vitamin E, at 50 mg/kg body weight. On the fifth day except for group I, all other group animals received 0.5 ml/kg body weight of CCl_4, intraperitoneally (Raja *et al.*, 2005b, 2006; Rai *et al.*, 2006). On the seventh day, all the animals were sacrificed by decapitation. Liver and kidney were removed, weighed and homogenized immediately with Teflon homogenizer, in ice chilled 10% KCl solution (10 mg/g of tissue). The suspension was centrifuged at 2,000 rpm at 4°C for 10 min and clear supernatant was used for the following estimation. Catalase was estimated by following the breakdown of hydrogen peroxide according to the method of Beer and Seizer (1952). SOD was assayed according to Misra and Fridovich (1972) based on the inhibition of epinephrine auto-oxidation by the enzyme. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content following the thiobarbituric acid method of Okhawa *et al.* (1979). Statistical analysis was carried out using the Student's *t*-test and the results were judged significant, at $P < 0.05$.

RESULTS

DPPH radical scavenging assay revealed the antioxidant potentials of HHME. The extract was found to scavenge more radicals than other extracts with IC_{50} at 5.82 ± 1.33 μg/ml. The value was found to be comparable to that of standard gallic acid as shown in Table 1. HHPE was found to be more active than other successive extracts in inhibiting nitric oxide radical, with IC_{50} at 220.97 ± 2.69 μg/ml. When subjected for *in vitro* TBARS assay, methanolic extract was found to be most potent than other extracts with IC_{50} at 49.78 ± 3.79 μg/ml (Table 1).

No significant behavioral change and no mortality was observed, when HHPE and HHCL were administered to mice up to the oral dose of 600 mg/kg for 24 h, and during this time no mortality was observed. HHEA and HHME showed no significant behavioral change and mortality, when administered to mice up to 800 mg/kg and 1,000 mg/kg respectively, for 24 h. Decreased level of catalase and SOD, together with a significant increase in the level of TBARS in both liver and

Table 1. *In vitro* antioxidant potentials of Indian *H. hookerianum*

Extract	IC_{50} (μg/ml) DPPH assay	IC_{50} (μg/ml) NO scavenging assay	IC_{50} (μg/ml) TBARS inhibition
HHPE	186.70 ± 4.68	220.97 ± 2.69	174.85 ± 4.63
HHCL	70.95 ± 4.85	375.09 ± 4.85	103.22 ± 2.66
HHEA	7.18 ± 1.35	510.98 ± 3.66	69.83 ± 2.75
HHME	5.82 ± 1.33	> 1000.00 (No activity)	49.78 ± 3.79
Gallic acid	1.38 ± 0.07	-	-
Quercetin	-	135.00 ± 3.61	21.42 ± 1.74

Results are expressed in IC_{50} (concentration required to inhibit 50% of free radicals) value of the mean ± S.E.M. All the *in vitro* experiments were carried out in triplicate. (IC_{50} value for methanolic extract in NO scavenging assay was found to be more than concentrations used in the studies)

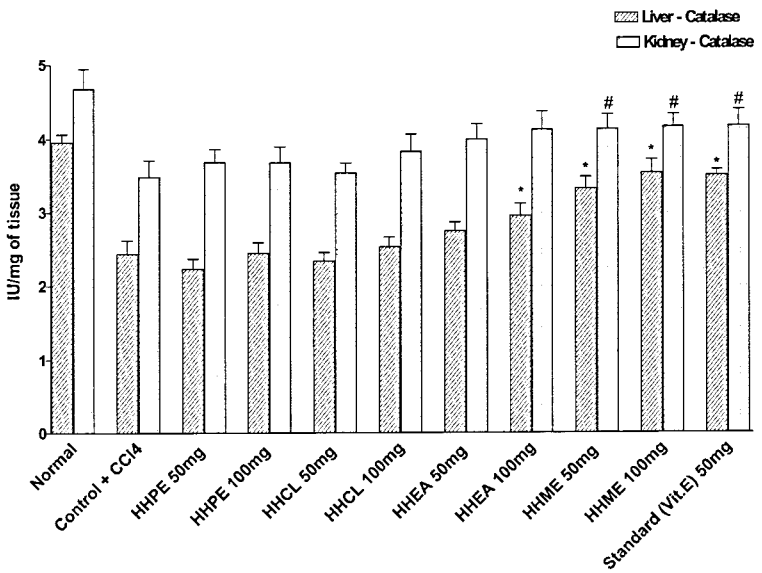

Fig. 1. Levels of catalase in liver and kidney of *H. hookerianum* extracts and CCl_4 treated animals. Levels of catalase was reduced in CCl_4 treated group, when compared to untreated group. The level was found to be increased in standard and extract treated groups. Results are mean ± S.E. (n = 6), $^{\#}P < 0.05$, $^{*}P < 0.01$, when compared with CCl_4 control group.

kidney, was observed in CCl_4 treated animals when compared with the untreated animals (Figs. 1 - 3). Administration of HHME at 50 and 100 mg/kg body weight for 4 days before CCl_4 treatment shows significant reversal of these changes towards the normal group, in both liver and kidney ($P < 0.05$), when compared to CCl_4 treated control. Significant increase in the levels of SOD and catalase were observed in both the liver and kidney by HHME treatment at 100 mg/kg body weight, when compared to CCl_4 treated control. Similarly at same tested dose it shows significant decrease in the level of TBARS. These changes by HHME at 100 mg/kg body weight treatment were comparable to that of standard vitamin E at 50 mg/kg.

DISCUSSION

The antioxidant defense mechanism includes free radicals scavenging enzymes like SOD and catalase generated in the body and chemical entities, which are capable of augmenting the activity of these

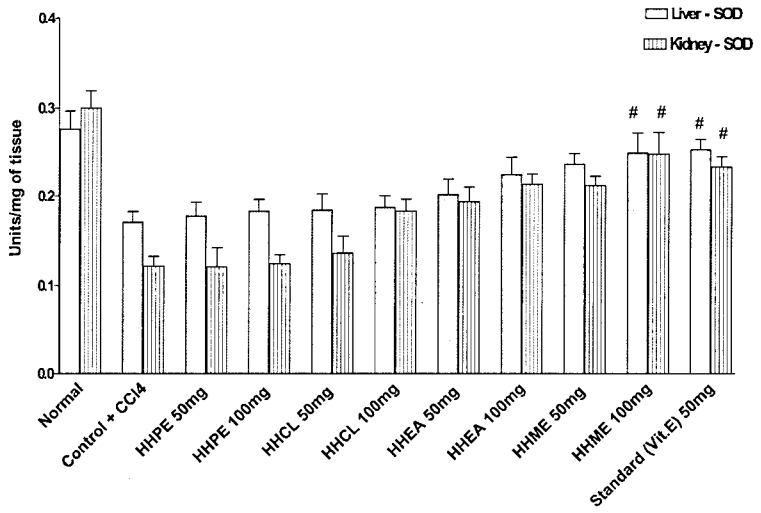

Fig. 2. Levels of SOD in liver and kidney of *H. hookerianum* extracts and CCl_4 treated animals. Levels of SOD was reduced in CCl_4 treated group, when compared to untreated group. As like catalase level, level of SOD was also found to be increased in standard and extract treated groups. Results are mean ± S.E. (n = 6), $^{\#}P < 0.05$, when compared with CCl_4 control group.

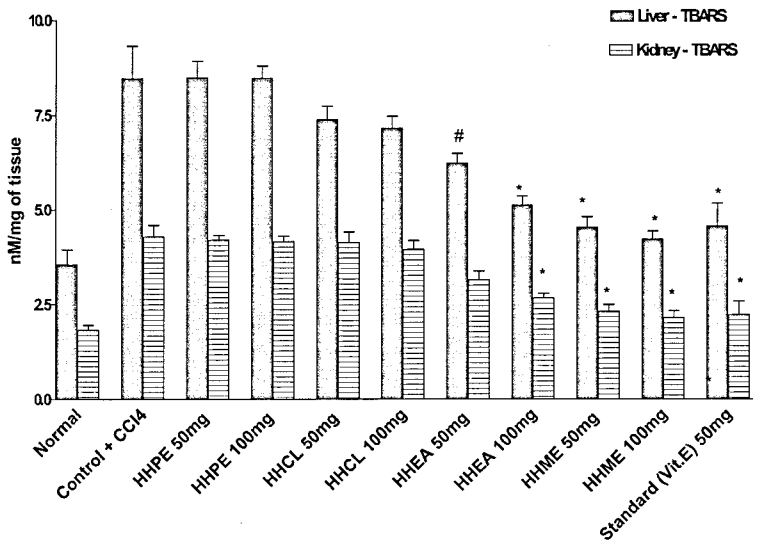

Fig. 3. Levels of TBARS in liver and kidney of *H. hookerianum* extracts and CCl_4 treated animals. Levels of TBARS were found to be increased in CCl_4 treated groups. The levels were reduced significantly in standard as well as ethyl acetate and methanolic extract treated groups. Results are mean ± S.E. (n = 6), $^{\#}P < 0.05$, $^{*}P < 0.01$, when compared with CCl_4 control group.

enzymes (Cheeseman and Scater, 1993). Antioxidant enzymes play an important role in body defense mechanism against harmful effects of the ROS and free radicals in biological systems (Halliwell and Gutteridge, 1989). Lipid peroxidation caused by ROS yields a wide range of cytotoxic products most of which are aldehydes, as exemplified by MDA (Yagi and Rastogi, 1979). It has been reported that antioxidants might, protect against CCl_4-induced toxicity through anti-peroxidation and induction of defense enzyme expression (Maurizio *et al.*, 1992). Potent antioxidant extracts and compounds

are known to increase the levels of catalase and SOD and decrease the level of TBARS in blood and tissues. Similar and significant reversals of these changes towards the normal were observed with the treatment of *H. hookerianum* which confirms its antioxidant properties.

Several other plants species from traditional systems of medicines have been reported to possess significant antioxidant potentials. Plants like *Withania somnifera, Bacopa monniera, Vitis vinifera* and *Mangifera indica* are reported to use in traditional system of medicines in treatment of different disorders, where ROS plays important role; these plants have been reported to possess significant antioxidant and hepatoprotective potentials. (Bhattacharya *et al.*, 2001; Anila and Vijayalakshmi, 2003; Rohini *et al.*, 2004). An Ayurvedic polyherbal formulation Liv.52 containing various herbs has been reported to have potential hepatoprotective activity (Saxena and Garg, 1979, 1981; Bardhan *et al.*, 1985).

Different *H.* species have also been used against inflammation, liver dysfunction, helminthiasis, and cardiac disorders where ROS generated by stress and other disorders plays a critical role in causing these diseases. Several *in vitro* and *in vivo* bioassays with different *H.* species have highlighted their significant role in inhibition of ROS when administered either in extract form or isolated bioactive compounds (Conforti *et al.*, 2002). Concentrations between 1 and 50 mg/ml of *H. perfomtum* extract effectively inhibited lipid peroxidation of rat brain cortex mitochondria induced by Fe^{2+}/ ascorbate or NADPH system (Benedi *et al.*, 2004). It has been reported that, the antioxidant potentials exhibited by *H.* species are almost similar to that of standard antioxidant á-tocopherol (Couladis *et al.*, 2002). Phytoconstituents like flavonoids, quercetin-3-O-galactoside, kaempferol-3-O-glycoside, (-) epicatechin isolated from different *H.* species have been reported to demonstrate potent antioxidant activity. Investigation on the antioxidant activity of I3, II8-biapigenin isolated from *H. perforatum* showed stronger protection against oxidation (Conforti *et al.*, 2002). Hyperforin a dianthrone from *H. perforatum*, possesses significant antioxidant potential. Antioxidant potentials of *H. perforatum* could be highlighted with a fact that diluted *H. perforatum* extract at a dilution of 1 : 10 and 1 : 20 exhibited significant super oxide dismution effect when tested on human tissue extract. This facilities *H.* administration by oral route for antioxidant effect where it gets metabolized by liver and then gets diluted in blood (Hunt *et al.*, 2001).

Under present studies, *in vitro* antioxidant screening using DPPH and nitric oxide methods showed strong antioxidant activity of HHME and HHCL respectively. In DPPH assay The IC_{50} values of the HHME were comparable to the standard gallic acid. The lipid peroxidative degradation of the bio-membrane is one of the principle causes of toxicity of CCl_4 (Kaplowitz *et al.*, 1986). This is evidenced by the elevation of TBARS and decrease in the activity of free radical scavenging enzymes, viz., SOD and catalase in the CCl_4 treated animals. SOD is the key enzyme in scavenging the superoxide radicals. Catalase is also another key enzyme in the scavenging, which helps in cleaning the radicals formed during incomplete oxidation (Halliwell and Gutteridge, 1989). In the present study, administration of the HHEA at 100 mg/kg body weight prior to CCl_4 treatment caused a significant increase in the level of SOD and catalase and a significant decrease in the level of TBARS when compared to CCl_4 treated control in both liver and kidney. The values at 100 mg/kg body weight treatment were comparable to that of vitamin E at 50 mg/kg, used as standard.

Lipid peroxidation is found to be an important pathophysiological event in a variety of diseases including hepatotoxicity, aging, cancer, diabetes, cardiovascular disorders and rheumatoid arthritis (Ajitha and Rajnarayana, 2001). Hence, current interest has focused on the potential role of antioxidants in the treatment and prevention of such diseases. Under the present study, the *in vitro* and *in vivo* antioxidant potentials of successive extracts of *H.*

hookerianum were investigated which further highlighted possible hepatoprotective activity. It can be concluded that *H. hookerianum* possess antioxidant and hepatoprotective properties as evidenced by the significant dose dependent increase in the level of catalase, SOD and decrease in the levels of TBARS. The *in vitro* studies also confirm the same. However, the detailed mechanisms are not fully understood and remain to be further resolved.

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REFERENCES

Ajitha M, Rajnarayana K. (2001) Role of oxygen free radicals in human diseases. *Indian Drugs* **38**, 545-554.

Anila L, Vijayalakshmi NR. (2003) Antioxidant action of flavonoids from *Mangifera indica* and *Emblica officinalis* in hypercholesterolemic rats. *Food Chem.* **83**, 569-574.

Anonymous. (1997) *The Wealth of India*, Vol. V, p. 154, PID CSIR, New Delhi.

Badami S, Moorkoth S, Rai SR, Kannan E, Bhojraj S. (2003) Antioxidant activity of *Caesalpinia sappan* heartwood. *Biol. Pharm. Bull.* **26**, 1534-1537.

Bardhan P, Sharma SK, Garg NK. (1985) In vitro effect of an Ayurvedic liver remedy on hepatic enzymes in carbon tetrachloride treated rats. *Indian J. Med. Res.* **82**, 359-364.

Beer RF Jr, Seizer TW. (1952) A spectrophotometric method for measuring breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **115**, 130-140.

Benedi J, Arroyo R, Romero C, Martin-Aragon S, Villar AM. (2004) Antioxidant properties and protective effects of a standardized extract of *Hypericum perforatum* on hydrogen peroxide-induced oxidative damage in PC12 cells. *Life Sci.* **75**, 1263-1276.

Bhattacharya A, Ghosal S, Bhattacharya SK. (2001) Anti-oxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J. Ethnopharmacol.* **74**, 1-6.

Cakir A, Mavi A, Yildirim A, Durn ME, Harmandar M, Kazaz C. (2003) Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. *J. Ethnopharmacol.* **87**, 73-83.

Cheeseman KH, Scater TF. (1993) Free radical in medicine. *Br. Med. Bull.* **49**, 479-724.

Conforti F, Statti GA, Tundis R, Menichini F, Houghton P. (2002) Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part. *Fitoterapia* **73**, 479-483.

Couladis M, Baziou P, Verykokidou E, Loukis A. (2002) Antioxidant Activity of Polyphenols from *Hypericum triquetrifolium* Turra. *Phytother. Res.* **16**, 769-770.

El-Sherbiny DA, Khalifa AE, Attia AS, Eldenshary Eel-D. (2003) *Hypericum perforatum* extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine. *Pharmacol. Biochem. Behav.* **76**, 525-533.

Gamez EJ, Luyengi L, Lee SK, Zhu LF, Zhou BN, Fong HH, Pezzuto JM, Kinghorn AD. (1998) Antioxidant flavonoid glycosides from *Daphniphyllum calycinum*. *J. Nat. Prod.* **61**, 706-708.

Garrat DC. (1964) *The Quantitative Analysis of Drugs*, Vol. 3, pp. 456-458, Chapman and Hall Ltd., Japan.

Gutteridge JM. (1993) Free radicals in disease processes: a compilation of cause and consequence. *Free Radic. Res. Commun.* **19**, 141-158.

alliwell B, Gutteridge JMC, (1989) *Free Radicals in Biology and Medicine*. 2nd ed., Clarendron Press, Oxford.

Halliwell B. (1990) How to characterize a biological antioxidant. *Free Radic. Res. Commun.* **9**, 1-32.

Halliwell B, Gutteridge JM, Cross CE. (1992) Free radicals, antioxidants and human diseases: where are we now? *J. Lab. Clin. Med.* **119**, 598-613.

Halliwell B, Gutteridge JMC. (1995) *Free Radicals in Biology and Medicine*. Oxford: Clarendon Press.

Hunt EJ, Lester CE, Lester EA. (2001) Effect of St. John's wort on free radical production. *Life Sci.* **69**, 181-190.

Hwang BY, Kim HS, Lee JH, Hong YS, Ro JS, Lee KS, Lee JJ. (2001) Antioxidant benzoylated flavan-3-ol glycoside from *Celastrus orbiculatus*. *J. Nat. Prod.* **64**, 82-84.

Kaplowitz N, Aw TY, Simon FR, Stolz A. (1986) Drug-induced hepatotoxicity. *Ann. Intern. Med.* **104**, 826-839.

Marcocci L, Packer L, Droy-Lefaix MT, Sekaki A, Gardes-Albert M. (1994) Antioxidant action of Ginkgo biloba extract EGb 761. *Meth. Enzymol.* **234**, 462-475.

Maurizio P, Leonarduzzi G, Biasi F, Albano E, Biocca ME, Poli G, Dianzani MU. (1992) Vitamin E dietary supplementation protects against carbon tetrachloride-induced chronic liver damage and cirrhosis. *Hepatology* **16**, 1014-1021.

Misra HP, Fridovich I. (1972) The role of superoxide anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **247**, 3170-3175.

Mukherjee PK, Suresh B. (2000) The evaluation of wound-healing potential of *Hypericum hookerianum* leaf and stem extracts. *J. Altern. Complement. Med.* **6**, 61-69.

Mukherjee PK, Saritha GS, Suresh B. (2001a) Antibacterial spectrum of *Hypericum hookerianum*. *Fitoterapia* **72**, 558-560.

Mukherjee PK, Suresh B, Verpoorte R. (2001b) CNS active potentials of some Hypericum species of India. *Phytomedicine* **8**, 331-337.

Mukherjee PK, Wahile A, Nazeer A, Rajan S. (2003) Hypericum Source of Natural Antimicrobials. *Orient. Pharm. Exp. Med.* **3**, 111-122.

Mukherjee PK. (2002) *Quality Control of Herbal Drugs - An Approach to Evaluation of Botanicals*, pp. 560-567, Business Horizons, New Delhi, India.

Mukherjee PK. (2003) Plant products with hypocholesterolemic potentials. *Adv. Food Nutr. Res.* **47**, 278-324.

Mukherjee PK, Verpoorte R. (2003) *GMP in Herbal Drugs*, pp 366-367, Eastern Publishers, Business Horizons Ltd, New Delhi, India.

Ohkawa H, Ohishi N, Yagi K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351-358.

Rai S, Wahile A, Mukherjee K, Saha BP, Mukherjee PK. (2006) Antioxidant activity of Nelumbo nucifera (sacred lotus) seeds. *J. Ethnopharmacol.* **104**, 322-327.

Raja S, Nazeer Ahamed KFH, Kumar V, Mukherjee K, Bandyopadhyay A, Mukherjee PK. (2005a) Antioxidant potential of Aerial part of Asclepias curassavica. Linn (Family - Asclepiadaceae). *Orient. Pharm. Exp. Med.* **5**, 92-99.

Raja S, Nazeer Ahamed KFH, Kumar V, Mukherjee K, Bandyopadhyay A, Mukherjee PK. (2005b) Exploring the Effect of *Asclepias curassavica* on Markers of Oxidative Stress in Rats. *Evidence Based Integr. Med.* **2**, 87-93.

Raja S, Ahamed KF, Kumar V, Mukherjee K, Bandyopadhyay A, Mukherjee PK. (2006) Antioxidant effect of *Cytisus scoparius* against carbon tetrachloride treated liver injury in rats. *J. Ethnopharmacol.* (In press).

Rajlakshmi D, Banerjee SK, Sood S, Maulik SK. (2003) *In-vitro* and *in-vitro* antioxidant activity of different extracts of the leaves of *Clerodendron colebrookianum* Walp in rats. *J. Pharm. Pharmacol.* **55**, 1681-1686.

Rohini G, Sabitha KE, Devi CS. (2004) *Bacopa monniera* Linn. extract modulates antioxidant and marker enzyme status in fibrosarcoma bearing rats. *Indian J. Exp. Biol.* **42**, 776-780.

Saxena A, Garg NK. (1979) Effect of Liv-52 on hepatic enzymes. *Indian J. Exp. Biol.* **17**, 662-664.

Saxena A, Garg NK. (1981) Effect of Liv-52 on membrane lipids in carbon tetrachloride induced hepatotoxicity in rats. *Indian J. Exp. Biol.* **19**, 859-862.

Sharma MK, Buettner GR. (1993) Interaction of vitamin C and vitamin E during free radical stress in plasma: an ESR study. *J. Free Radic. Biol. Med.* **14**, 649-653.

Sundararajan R, Haja NA, Venkatesan K, Mukherjee K, Saha BP, Bandyopadhyay A, Mukherjee PK. (2006) Cytisus scoparius link-a natural antioxidant. *BMC Complement. Altern. Med.* **16**, 6 (In press).

Trocino RA, Akazawa S, Ishibashi M, Matsumoto K, Matsuo H, Yamamoto H, Goto S, Urata Y, Kondo T, Nagataki S. (1995) Significance of glutathione depletion and oxidative stress in early embryogenesis in glucose-induced rat embryo culture. *Diabetes* **44**, 992-998.

Upton. (1997) St. John's Wort, American Herbal Pharmacopoeia and Therapeutic Compendium, p.12, Pittsburgh, PA, U.S.A.

Visioli F, Keaney JF, Halliwell B. (2000) Antioxidants and cardiovascular disease; panaceas or tonics for tired sheep? *Cardiovasc. Res.* **47**, 409.

Yagi K, Rastogi R. (1979) Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351.