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Antioxidant effect of *Cytisus scoparius* against carbon tetrachloride treated liver injury in rats

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Abstract

The study was aimed to investigate the antioxidant activity of *Cytisus scoparius* L. (Family: Leguminosae) on CCl_4 (carbon tetrachloride) treated oxidative stress in Wistar albino rats. CCl_4 injection induced oxidative stress by a significant rise in serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), lactate dehydrogenase (LDH) and thiobarbituric acid reactive substances (TBARS) along with reduction of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-*s*-transferase (GST) and glutathione reductase (GRD). Pretreatment of rats with different doses of plant extract (250 and 500 mg/kg) significantly lowered SGOT, SGPT, LDH and TBARS levels against CCl_4 treated rats. GSH and hepatic enzymes like SOD, CAT, GPx, GRD, and GST were significantly increased by treatment with the plant extract, against CCl_4 treated rats. The activity of extract at the dose of 500 mg/kg was comparable to the standard drug, silymarin (25 mg/kg). Based on these results, it was observed that *Cytisus scoparius* extract protects liver from oxidative stress induced by CCl_4 in rats and thus helps in evaluation of the traditional claim on this plant.

Keywords: *Cytisus scoparius*; Antioxidant; CCl_4 treated; Oxidative stress; Hepatoprotective

1. Introduction

Reactive oxygen free radicals have been known to produce tissue injury through covalent binding and lipid peroxidation and have been shown to augment fibrosis as seen from increased collagen synthesis (Geesin et al., 1990). Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues (Thresiamma and Kuttan, 1996). In recent years, the clinical importance of the herbal drugs has received considerable attention. Free radicals may also be a contributory factor in a progressive decline in the function of the immune system (Pike and Chandra, 1995). Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes. The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and indirectly glutathione reductase (GRD). Their roles as protective enzymes are well known and have been investigated extensively with in vivo models.

Several medicinal plants have been screened based on the integrative approaches on drug development from Ayurveda (Mukherjee and Wahile, 2006). *Cytisus scoparius* Link (Family: Leguminosae) also called as *Sarothamnus scoparius* is a well known plant in Ayurveda. This plant is used as diuretic, hypnotic and sedative (Siegel, 1976), anti-diabetic (Rebelo and Castro, 1998) and also as hepatoprotective (Rivera and Obon, 1995). *Cytisus scoparius* contains the flavone like 6"-*O* acetyl scoparin (Brum-Bousquet et al., 1977), flavonals, namely, rutin, quercetin, quercitrin, iso rhamnetin and kaempferol (Brum-

Abbreviations: BW, body weight; CAT, catalase; CCl_4, carbon tetrachloride; EDTA, ethylenediamine tetra acetic acid; GPx, glutathione peroxidase; GRD, glutathione reductase; GSH, reduced glutathione; GST, glutathione-*s*-transferase; IU, international unit; LDH, lactate dehydrogenase; LPO, lipid peroxidation; mU, milliUnit; NADH, nicotinamide adenine dinucleotide reduced disodium salt; NADPH, nicotinamide adenine dinucleotide phosphate; nM, nanomole; s, seconds; SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase; SNPS, school of natural product studies; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances

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Bousquet and Paris, 1974) and some isoflavones like genistein and sarothamnoside (Viscardi et al., 1984). Three carotenoids of chrysanthemaxanthin, xanthophyll and xanthophyllepoxide are also reported to be present in the plant (Chopra et al., 1956). Quinolizidine alkaloids, namely, spartein, sarothamine and lupanine (Wink et al., 1981) and benzenoid compounds like tyramine, hydroxy tyramine, phenyl ethanol and cresol (Kurhara and Kikuchi, 1980) have also been reported to be present in the plant. Plants containing flavonoids have been reported to possess antioxidant properties (Raj and Shalini, 1999). The present study was undertaken to investigate the antioxidant activity of hydroalcoholic extract of *Cytisus scoparius* in CCl_4 (carbon tetrachloride)-treated liver injury in rats.

2. Materials and methods

2.1. Plant material

Fresh aerial parts of *Cytisus scoparius* was collected from Nilgiri hills, Tamilnadu region and authenticated through Government Arts College, Ooty. Voucher specimen (SNPS-011/2003-2004) has been retained in the School of Natural Product Studies (SNPS), Jadavpur University, India.

2.2. Extraction

The aerial part of *Cytisus scoparius* was shed dried at room temperature and reduced to coarse powder. The powder was extracted with mixture of ethanol:water (7:3 ratio) at 60 °C. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in a vacuum desiccator for further use.

2.3. Chemicals

All chemicals were analytical grade and chemicals required for all biochemical assays were obtained from Sigma Chemicals Co., USA.

2.4. Animals

Wistar albino rats (200–250 g) and mice (20–25 g) of either sex were maintained under standard environmental conditions and had free access to feed and water. Experiments on animals were performed based on animal ethics guidelines of Institutional Animal Ethics Committee.

2.5. Behavioural and toxic effects

The acute oral toxicity study was evaluated in mice according to the method of Litchfield and Wilcoxon (1949). Five groups of 10 mice were administered with 250, 500, 1000, 2000 and 4000 mg/kg, of the *Cytisus scoparius* extract orally, while one group with the same number of mice served as a control. The animals were observed continuously for 1 h for any gross behavioural changes, symptoms of toxicity and mortality if any and intermittently for the next 6 h and then again, 24 h after dosing with plant extract.

2.6. Experimental treatments

Seven days after acclimatization, the rats were divided into five groups with six rats each. Group 1 served as control and was given the vehicle alone (propylene glycol, 5 mL/kg BW, per day p.o.) for 7 days. Group 2 animals received single dose of equal mixture of carbon tetrachloride and olive oil (50%, v/v, 5 mL/kg i.p.) on the 7th day. Groups 3 and 4 animals were treated with plant extract at dose level of 250 and 500 mg/kg per day p.o., respectively, for 7 days and on 7th day; a single dose of carbon tetrachloride (5 mL/kg i.p.) was administered. Group 5 animals were treated with standard drug, silymarin (25 mg/kg per day p.o.) for 7 days and on 7th day, a single dose of equal mixture of carbon tetrachloride and olive oil was administered (Rai et al., 2006).

2.7. Preparation of serum from blood

After 24 h, animals were sacrificed by chloroform anesthesia. Blood was collected by heart puncture. The blood samples of each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at $600 \times g$ for 15 min and analyzed for various biochemical parameters including serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) and lactate dehydrogenase (LDH) (Ahamed et al., 2003).

2.8. Preparation of liver homogenate

Hepatic tissues were homogenized in KCl [10 mM] phosphate buffer (1.15%) with ethylene-diamine tetra acetic acid (EDTA; pH 7.4) and centrifuged at $12{,}000 \times g$ for 60 min. The supernatant was used for assay of the marker enzymes (glutathione peroxidase, glutathione-*s*-transferase, glutathione reductase, superoxide dismutase and catalase), reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

2.9. Biochemical estimation of markers of oxidative stress

2.9.1. Effect of treatment with carbon tetrachloride on the level of lactate dehydrogenase activity

Lactate dehydrogenase activity was estimated in serum by the method of Kornberg (1955). The reaction mixture consisted of 0.1 mL of nicotinamide adenine dinucleotide reduced disodium salt (NADH, 0.02 M), 0.1 mL of sodium pyruvate (0.01 M), 0.1 mL of serum and made up to 3 mL with sodium phosphate buffer (0.1 M; pH 7.4). The changes in the absorbance were recorded at 340 nm at 30 s interval each for 3 min and the enzyme activity was calculated using a molar extinction coefficient of $6.220\ M^{-1}\ cm^{-1}$ and was expressed as nanomoles NADH oxidized/min/mg protein.