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Full Length Research Paper

Prevention of CCl_4 induced adrenal oxidative stress in rat by *Sonchus asper*

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Sonchus arvensis **is used as salad, vegetable and as a human diet in Pakistan, traditionally used in the treatment of various ailments. This study was designed to investigate the mathanol extract of *S. arvensis* was evaluated against CCl_4-induced adrenal oxidative stress in rat. The extract was administered to rats after 48 h of CCl_4 treatment (3 ml/kg b.w., 30% in olive oil) twice a week for 4 weeks. CCl_4 induction in rats caused reduction of antioxidant enzymes (CAT, SOD, GST, GSR, GSH-Px, and QR) while increased lipid peroxidation (TBARS) which are significantly *(P<0.01)* reversed by mathanol fraction of *S. arvensis* (CAT, SOD, GST, GSR, GSH-Px, and QR) enzymes. It is inferred that *S. arvensis* efficiently protects adrenal injuries induced by CCl_4 in rats, possibly through antioxidant effects of bioactive compounds present in the fraction.**

Key words: *Sonchus asper*, adrenal gland, antioxidant enzymes, carbon tetrachloride, glutathione reduced (GSH), thiobarbituric acid (TBARS).

INTRODUCTION

Carbon tetrachloride (CCl_4) is a potent hepatotoxic agent causing hepatic necrosis and nephrosis, and is widely used in animal models for induction of acute and chronic injury (Khan et al., 2009; Khan et al., 2010a; 2010b). It has been found that metabolism of CCl_4 involves the production of highly fatal trichloromethyl radical ($CCl_3^{\bullet}$) and proxy trichloromethyl ($^{\bullet}OOCCl_3$) free radicals through P450 bioactivation (Weber et al., 2003; Khan and Ahmed, 2009). CCl_4 is able of causing lipid peroxidation and decreases activity of antioxidant enzymes (Adewole et al., 2007). Medicinal plants, their fractions and bioactive compounds play crucial role in detoxification of such toxins and scavenge free radicals (Sahreen et al., 2010; Khan et al., 2010c). With increasing recognition of herbal medicine as an alternative form of healthiness, the objective of this study was to evaluate the antioxidant properties of *Sonchus arvensis* against the carbon tetrachloride (CCl_4) induced adrenal injuries in Sprague-Dawley male rats.

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Abbreviation: CAT, Catalase; **SOD,** super oxide dismutase; **GST,** glutathione-S-transferase; **GSR,** glutathione reductase; **GSH-px,** glutathione peroxidases; **QR,** quinone reductase; **GSH,** glutathione reduced; **TBARS,** thiobarbituric acid.

MATERIALS AND METHODS

Plant collection and extraction

Sonchus asper was collected from District Bannu (NWFP), identified, shade dried, grinded mechanically and extracted with methanol. Methanol extract was evaporated and used for *in vivo* investigation.

Experimental design and animal treatment

Six week old, 30 male albino rats (190 to 200 g) were purchased from National Institute of Health Islamabad and were kept in ordinary cages at room temperature of 25±3°C with a 12 h dark/light cycle as per the study protocol which was approved by Ethical Committee of Quaid-i-Azam University Islamabad. To study the antioxidant assets of *Sonchus asper*, male albino rats were equally divided into 5 groups (6 rats). Group 1 (control) have free access to food materials. Group II received CCl_4 3 ml/kg intraperitoneally (30% in olive oil, Monday and Thursday). Group III

Table 1. Effect of SAME on adrenal gland oxidative stress parameters.

Treatment	CAT (U/min)	SOD (U/mg protein)
Control	6.5±0.75++	12.0±0.67++
DMSO + Olive oil	6.4±0.47++	13.0±0.34++
3 ml/kg CCl_4	3.7±0.40**	7.5±0.64**
100 mg/kg SAME + CCl_4	5.9±0.76*++	10.3±0.49**++
200 mg/kg SAME + CCl_4	6.3±0.37++	12.7±0.67++
200 mg/kg SAME alone	6.6±0.45++	13.7±0.64++

Mean ± SE (n = 6 number), *, ** indicate significance from the control group at P<0.05 and P<0.01 probability level, +, ++ indicate significance from the CCl_4 group at P<0.05 and P<0.01 probability level.

and IV were given orally 100; 200 mg/kg b.w. (in DMSO), *Sonchus asper* methanol extracts (SAME) after 48 h of CCl_4 treatment (Wednesday and Saturday) as previously described. Groups V received only SAME in DMSO at a dose of 200 mg/kg b.w. (Wednesday and Saturday). After 24 h of the last treatment, all the animals were weighted, sacrificed and their blood were collected, isolated adrenal gland, was weighted and perfuse in ice-cold saline solution for further study.

Assessment of antioxidant enzymes

Adrenal tissue were homogenized in 10 volume of 100 mmol KH_2PO_4 buffer containing 1 mmol EDTA (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for the following experiments as described subsequently. Protein concentration of the supernatant of liver tissue was determined using crystalline BSA as standard. CAT activities were determined by the method of Chance and Maehly (1955) and SOD activity of lungs was estimated by the method of Kakkar et al. (1984).

Assessment of lipid peroxidation enzymes

Induction of lipid peroxidation by CCl_4 and its protection by the *Sonchus asper* extract was determined by the estimation of various enzyme activities and thiobarbituric acid reactive substances (TBARS) contents. Activity of glutathione-S-transferase activity was assayed by the method of Habig et al. (1974), glutathione reductase activity was determined by method of Carlberg and Mannervik (1975), glutathione peroxidase activity was assayed by the method of Mohandas et al. (1984) and quinone reductase was determined by the method of Benson et al. (1980).

Estimation of reduced glutathione and lipid peroxidation contents (TBARS)

Reduced glutathione was estimated by the method of Jollow et al. (1974) while TBARS contents were find out by the modified method of Iqbal et al. (1996).

Statistical analysis

To determine the treatment effects one way analysis of variance was carried by computer software SPSS 13.0. Level of significance among the various treatments was determined by LSD at 0.05% level of probability.

RESULTS

Effect of extract on adrenal gland oxidative stress parameters

Free radical of CCl_4 causes degradation of antioxidant enzymes namely; SOD and CAT. Treatment of CCl_4 extensively decreased *(P<0.01)* the activity of CAT and SOD. Supplementation of SAME to rats intoxicated with CCl_4 significantly reimbursed *(P<0.01)* and normalized their activity near to control rats proving its role in detoxification. Non significant changes (P>0.05) were found by feeding of mathanol fraction alone (Table 1).

Antioxidant polyphenolic compounds play key role in detoxification of reactive oxygen species (ROS) and maintain cellular balance. Effects of administration of SAME in rats are shown in Table 2. CCl_4 administration in rats significantly decreased *(P<0.01)* the activity of phase II metabolizing enzymes including GST, GSH-Px, GSR and QR. Treatment of rats to SAME intoxicated with CCl_4 significantly recovered *(P<0.01)* the activity to control rats while non significant changes (P>0.05) were found by only feeding of mathanol fraction alone (Table 2).

Effects of SAME on TBARS and GSH contents

GSH play important role in metabolism of free radicals and try to decreases lipid peroxidation TBARS contents. Administration of CCl_4 depleted *(P<0.01)* GSH contents and inversely increased *(P<0.01)* TBARS (Table 3). Supplementation of SAME reversed the contents and protects rat from *(P<0.01)* lipidperoxidation by various doses, however, 200 mg/kg shows comparatively more potency than other groups while non significant (P>0.05) changes were found with methanol extract of *Sonchus asper* alone.

Table 2. Effect of SAME on adrenal gland oxidative stress parameters.

Treatment	GST (nmol/ min/ mg protein)	GSH-Px (nmol/ min/ mg protein)	GSR(nmol/ min/ mg protein)	QR (nmol/ min/ mg protein)
Control	88.0±5.2++	45.0±2.8++	109.3±3.1++	123.0±4.7++
DMSO + Olive oil	86.3±4.1++	47.0±3.0++	113.6±4.3++	120.0±9.7++
3 ml/kg CCl_4	48.8±4.7**	26.3±2.89**	60.0±3.1**	69.0±6.9**
100 mg/kg SAME + CCl_4	70.3±5.47*++	39.3±3.4**++	82.1±4.1**++	96.6±4.3**++
200 mg/kg SAME + CCl_4	86.8±6.0++	42.5±4.27++	105.7±3.9++	111.5±4.9++
200 mg/kg SAME alone	89.7±8.8++	49.8±3.0++	109.3±6.7++	125.0±5.0++

Mean ± SE (n = 6 number), *, ** indicate significance from the control group at P<0.05 and P<0.01 probability level, +, ++ indicate significance from the CCl_4 group at P<0.05 and P<0.01 probability level.

Table 3. Effects of SAME on GSH and TBARS contents in rat adrenal gland.

Treatment	GSH (mol/ g tissue)	TBARS (nmol/ min/ mg protein)
Control	1.01±0.021++	14.7±1.6++
DMSO + Olive oil	1.1±0.005++	15.0±1.4++
3 ml/kg CCl_4	0.56±0.006**	24.3±2.57**
100 mg/kg SAME + CCl_4	1.0±0.015**++	16.7±2.29**++
200 mg/kg SAME + CCl_4	1.02±0.011++	14.3±1.77++
200 mg/kg SAME alone	1.08±0.018++	15.3±1.86++

Mean ± SE (n = 6 number), *, ** indicate significance from the control group at P<0.05 and P<0.01 probability level, +, ++ indicate significance from the CCl_4 group at P<0.05 and P<0.01 probability level.

DISCUSSION

A number of drugs, toxic industrial chemicals and viral infection have been reported to cause severe tissue injuries, which sometimes become difficult to be managed by medical therapies. It is important to evaluate plant extracts that can be used for better treatment of the hepatic failure due to severe oxidative stress and necrosis (Toklu et al., 2008). Antioxidant enzymes namely: SOD, CAT and GSH-Px compose a communally compassionate set of antioxidant defense against reactive oxygen species (Halliwell and Gutteridge, 1999; Khan and Ahmed, 2009; Khan et al., 2009). Our data revealed that CCl_4-treatment notably decreased the activities of catalase (CAT), superoxide dimutase (SOD), GSH-Px, GST, GSR and QR in pancreatic tissue. These enzymes play an important role in detoxification of reactive oxygen species (ROS) that produced metabolism of different xenobiotics in hepatic tissues. Supplementation of various doses markedly erased the toxicity of CCl_4 and the enzymatic activities. Repairing effects of different plant metabolites on these enzymes against the toxicity of CCl_4 have also been documented in various studies (Manna et al., 2007; Khan et al. 2009, 2010a, b). Data of the present study showed that CCl_4 significantly decreased the GSH while increased the TBARS contents as compared to the control. Administration of extract significantly increased the GSH contents and recovered the MDA and TBARS contents might be due to presence of phenolic and polyphenolic flavonoids. Similar results were reported by co-administration of *Digera muricata* extract against oxidative stress of CCl_4 (Khan and Ahmad, 2009). Other investigations have shown that antioxidants such as ascorbic acid, vitamin E and garlic, which markedly reduced lipid peroxidation through reduction of free radical levels, have been found to offer protection against CCl_4-induced hepatotoxicity (Alpinar et al., 2009).

Conclusion

This study revealed that mathanol fraction of *Sonchus arvensis* recovered the enzymatic alteration and repaired cellular injuries, provides scientific evidence in favour of its pharmacological use in adrenal dysfunction due be the presence of bioactive compounds.

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