



Journal of
**Pharmacology and
Toxicology**

ISSN 1816-496X



Academic
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Antioxidant Activity of *Chamomile recutita* Capitula Methanolic Extracts against CCl₄-Induced Liver Injury in Rats*

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Abstract: Antioxidant activity of Methanolic Extract (MET) of capitula of *Chamomile recutita* was investigated in rats by inducing liver injury with CCl₄. The MET exhibited significant antioxidant activity by showing increased levels of Glutathione Peroxidase (GPX), Glutathione-S-Transferase (GST), Glutathione Reductase (GRD), Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione (GSH). The decreased level of Lipid Peroxidation (LPO) has also been shown. The MET, at a dose level of 300 mg kg⁻¹ showed significant antioxidant activity against CCl₄ induced liver injury in rats. MET of *C. recutita* had been shown a potent and prominent free radical scavenging activity and CCl₄-intoxicated liver recovery towards normalcy in the methanol extract co-administered rats revealed the effectiveness of *C. recutita* in combating oxidation stress due to hepatic damage. Thus, the MET possess antioxidant activities with combined hepatoprotective action has a synergistic effect to stabilize cellular membrane or antiperoxidase activity.

Key words: Antioxidant, carbon tetra chloride, *Chamomile recutita*, glutathione, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, hepatoprotection, lipid peroxidation

Introduction

Free radicals such as Reactive Oxygen Species (ROS), halogenated hydrocarbons, etc., have been shown to modify biological molecules, which may result in various pathological conditions (Gupta *et al.*, 1992). In recent years, many plants have been screened for their antioxidant potential (Achuthan *et al.*, 2003; Aniya *et al.*, 2002; Jose and Kuttan, 1995; Lin *et al.*, 1995; Shylesh and Padikkala, 1999). However, deleterious effects of free radicals remain a problem. Thus, additional natural products need to be evaluated for their antioxidant potential.

The liver is the first organ to encounter the ingested nutrients, drugs and environmental toxicants that enter the hepatic portal blood from the digestive system. Liver function can be detrimentally-altered injury resulting from acute or chronic exposure to toxicants. These free radicals and ROS initiate lipid peroxidation and liver damage (Slater, 1984). Since carbon tetrachloride (CCl₄) is biotransformed to a trichloromethyl radical (CCl₃•) and then the peroxy-radical (CCl₃OO•) by the cytochrome P450 system in liver injury (McCay *et al.*, 1984; Recknagel, 1983; Slater, 1984), this compound has been used for the evaluation of free radical scavenging action *in vivo*.

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*Originally Published in *Journal of Pharmacology and Toxicology*, 2006

Chamomile recutita (cv. Prashant) L. Rousch, (family *Asteraceae*), popularly known as Chamomile is a reputed medicinal and aromatic plant used in both traditional and modern system of medicine. It is an ingredient of several traditional, Unani and Homeopathy medicinal preparations (Das *et al.*, 1998, 1987, 1996; Mann and Stabs, 1986; Ramaswamy *et al.*, 1988; Reichling *et al.*, 1979; 1983). The oil obtained from the capitula, is a natural source of 'blue oil' (chamazuline) and flavonoids, which are used in pharmaceuticals, perfumery and cosmetic industries. The plant is well known for its anti-inflammatory, wound healing, antiseptic and spasmolytic activity since the ancient times (Merikli, 1990).

Therefore the present study has been undertaken to investigate the antioxidant activity of MET of the capitula of *C. recutita* in CCl₄ intoxicated liver injury in rats.

Materials and Methods

Plant Material

The fresh capitula of *C. recutita* (1 kg) was collected from the University campus in the month of March 2004 and plant was identified and authenticated by the ex-director Dr. Sushil Kumar, Central Institute of Medicinal and Aromatic Plant Lucknow, India.

Preparation of Methanolic Extract

The freshly collected capitula of *C. recutita* were shade dried and coarsely powdered. The powder was then successively extracted with methanol using Soxhlet extractor. After drying the methanolic extract under reduced pressure using a rotary evaporator and kept under refrigeration. The extract was administered to the animals as suspension in propyleneglycol.

Animals

The experiments were carried out in male albino rats (150-200 g). They were maintained under control conditions of light and temperature 25±1°C, 60-70% relative humidity in the animal house of Institute of Pharmacy, Bundelkhand University, Jhansi. Food pellets (DRDE, Gwalior) and tap water were provided ad libitum. For experiment, animals were kept fasting overnight but were allowed free access to water. Six animals in each group were used in all sets of experiments. The animal experiments were performed according to the rules and regulations of Animal Ethical Committee, Government of India.

CCl₄ Toxicity

The adult albino rats (male) were divided into five groups of six animals each. Group I, received only propyleneglycol (2 mL kg⁻¹ per day, i.p.) for ten days and served as a control. Group II, CCl₄ in olive oil (1:9) (v/v) and administered intra peritoneally (final concentration: 200 µL kg⁻¹) up to the tenth day. Group III and IV, animals were treated with MET at doses of 150 and 300 mg kg⁻¹ per day p.o., respectively, for ten days. On the tenth day, a single dose of CCl₄ and olive oil (1:9) (v/v) was given. Group V, animals were treated with the silymarin (25 mg kg⁻¹ per day p.o.) for ten days and on the tenth day, a single dose of CCl₄ and olive oil (1:9) (v/v) was administered.

All animals sacrificed by cervical decapitation under light ether anesthesia on the eleventh day. After sacrifice, immediately, the liver was dissected out, washed in the ice-cold saline. The homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.5) containing 10 mM EDTA. The homogenate was centrifuged at 18000 xg. The supernatant was used for the assay of marker enzymes namely

Glutathione Peroxidase (GPX) and Glutathione-S-Transferase (GST) and Glutathione Reductase (GRD) by reported methods (Necheles *et al.*, 1968; Habig *et al.*, 1974; Dubler and Anderson, 1981). The activities of Superoxide Dismutase (SOD) and Catalase (CAT) were determined by Misra and Fridovich (1972) and Bergmeyer *et al.* (1994), respectively. LPO was estimated by the methods of Ohkawa *et al.* (1979).

Reduced glutathione (GSH) content in blood was measured by the method of Beutler *et al.* (1974) after some modification. This method is based on the development of yellow colour when 5,5'-dithiobis (2-nitro benzoic acid) (Ellmann's reagent DTNB) is added to sulphhydryl compounds.

The total protein was estimated by the Bio-Red micro assay modification of the Bradford (1976) using bovine serum albumin as standard.

Statistical Analysis

Each treatment was analyzed and standard deviation (SD) was calculated. Statistical analysis was performed using the student t-test ($p < 0.05$ was considered statistically significant).

Results

Glutathione Peroxidase (GPX)

GPX activity in liver was significantly ($p < 0.05$) decreased in CCl_4 -treated animals compared to control. The MET treatment (300 mg kg^{-1} dose level) significantly increased ($p < 0.05$) the GPX level as compared to CCl_4 -treated animals. However, MET (150 mg kg^{-1}) showed no significant increase in GPX levels in liver homogenate as compared to CCl_4 intoxicated animals. Silymarin (25 mg kg^{-1} p.o.) treated animals also showed significant ($p < 0.05$) increase of GPX level in the liver homogenate compared with CCl_4 treated animals (Table 1).

Glutathione-S-Transferase (GST)

In the present study, GST activity in liver was significantly decreased ($p < 0.05$) in CCl_4 treated animals as compared to control. The treatment of MET of *C. recutita* at 300 mg kg^{-1} dose showed significant increase ($p < 0.05$) in GST as compared with CCl_4 treated animals. However, low dose of MET (150 mg kg^{-1}) showed no significant increase in GST in liver homogenate as compared to CCl_4 treated group. A significant increase ($p < 0.05$) of GST in the liver homogenate of silymarin treated animals was observed (Table 1).

Glutathione Reductase (GRD)

GRD activity was significantly decreased ($p < 0.05$) in CCl_4 treated animals when compared to control. Further, a significant increase ($p < 0.05$) in GRD level was observed in MET (300 mg kg^{-1}) treated animals as compared to CCl_4 treated animals. There was no significant increase by MET (150 mg kg^{-1}) in the GRD, when compared with CCl_4 treated animals. A significant increase ($p < 0.05$) in the level of GRD was observed in liver homogenate of silymarin treated animals (Table 1).

Superoxide Dismutase (SOD)

SOD activity was significantly decreased in CCl_4 treated animals as compared with control. The MET (300 mg kg^{-1}) showed significant increase ($p < 0.05$) in SOD as compared to CCl_4 treated animals. A low dose (150 mg kg^{-1}) showed no significant increase in SOD level. Silymarin treated animals also showed significant ($p < 0.05$) increase in SOD as compared to CCl_4 treated animals (Table 1).

Table 1: Antioxidant activity of methanolic extract of capitula of *C. recutita* against CCl₄-induced liver damage

Biochemical Parameters	Control	CCl ₄ treated group	Methanolic extract (MET) of <i>C. recutita</i> (mg kg ⁻¹) + CCl ₄		Silymarin (25 mg kg ⁻¹) + CCl ₄
			150	300	
GPX (nmol of GSH oxidized/min/mg protein)	456.3±19.6	159.2±18.6	295.2±13.6	392.6±17.2	426.5±7.6
GST (nmole of CDND conjugate form/min/mg protein)	319.9±22.5	136.9±6.5	246.9±10.5	289.5±4.5	299.6±12.5
GRD (nmole of GSSG utilized /min/mg protein)	35.9±1.1	8.9±0.5	18.6±1.6	29.6±2.3	31.6±1.6
SOD (kat/gm protein)	99.5±6.5	35.6±2.5	56.5±3.4	85.7±4.9	92.9±8.8
CAT (nmole of H ₂ O ₂ decomposed/min/mg protein)	225±8.6	39±3.8	167±9.9	1.89±3.8	209±4.6
LPO (nmole of MDA /mg protein)	3.9±0.3	14.6±1.6	8.2±0.5	4.6±0.2	4.2±0.6
GSH (µgm/mg protein)	7.63±1.36	1.23±0.12	5.13±1.10	6.89±1.31	7.26±2.56

Catalase (CAT)

CAT level was significantly decreased ($p < 0.05$) in CCl₄ treated animals as compared to control. The MET (300 mg kg⁻¹) showed significant increased ($p < 0.05$) CAT in liver homogenate when compared to CCl₄ treated animals. The MET at 150 mg kg⁻¹ dose showed no significant increase of CAT, when compared with CCl₄ treated animals. Silymarin treated animals also showed significant ($p < 0.05$) increase of CAT than CCl₄ treated animals (Table 1).

Lipid Peroxidation (LPO)

LPO level significantly increased ($p < 0.05$) in CCl₄ treated animals as compared to control. MET of 300 mg kg⁻¹ showed significant ($p < 0.05$) decrease in LPO as compared with CCl₄ treated animals, while low dose (150 mg kg⁻¹) showed no significant decrease in LPO in liver homogenate as compared with CCl₄ treated animals. A significant decline ($p < 0.05$) in LPO was observed in silymarin treated animals (Table 1).

Glutathione (GSH)

GSH constitutes the first line of defense against free radical. Reduction in liver GSH and decrease in GPX activity in CCl₄-treated rats as observed (Table 1).

Discussion

The toxic effect of CCl₄ *in vivo* is well known to be mediated through radical reactions. The CCl₃O* and/or CCl₃OO* radicals produced as a result of the metabolic conversion of CCl₄ is reported to initiate peroxidation of lipid membrane causing tissue damage. Liver is the primary site of action of CCl₄ and the most important organ concern with the biochemical activities in the human body. It is well established that hepatotoxicity by CCl₄ is due to enzymatic activation to release CCl₃* radical in free state which in turn disrupts the structure and function of lipid and protein macromolecule in the membrane of the cell organelles (Mujumdar *et al.*, 1998).

GPX play a pivotal role in H₂O₂ catabolism (Eaton, 1991) and the detoxification of endogenous metabolic peroxides and hydroperoxides, which catalyzes GSH (Floka, 1971). GPX activity was significantly decreased after CCl₄ treatment as compared to control. After pretreatment with MET, the GPX activity was increased, is due to antioxidant activity by scavenging/detoxifying the endogenous metabolic peroxides generated after CCl₄ injury in the liver tissues.

It has been reported that GST plays a physiological role in initiating the detoxification of potential alkylating agents. This enzyme protect against LPO by elevating the conjugation of toxic electrophiles with GSH (Jakoby, 1988). GST activity was significantly reduced in CCl₄-induced animals while just reserve was observed after the treatment of MET. This may be attributed to a direct action of the extract on the hepatic GST activation.

Reduced glutathione is essential to maintain the structural and functional integrity of the liver. The maintenance of the cellular GSH level depends upon activity of GRD and NADH (Meister and Anderson, 1983). We found that in CCl₄-intoxicated animals, GRD activity decreased, while intern lower GSH content in liver. Treatment with a MET of *C. recutita* increased the activity of GRD as well as the GSH level of the liver. Thus, an increase in GRD activity implies that MET protect the liver from oxidation damage by GSH regenerated from its oxidized form (GSSG).

In the present study, the enhanced lipid peroxidation (LPO) expressed in terms of thiobarbituric acid reacting substances (TBARS) and significantly reduced activity of superoxide dismutase (SOD) and catalase (CAT) observed in CCl₄ treated animals (Table 1) confirms the hepatic damage to the CCl₄. The level of LPO is a measure of membrane damage as well as alteration in structure and function of cellular membrane. The lipid peroxidation (LPO) expressed in terms of level of thiobarbituric acid (TBARS) (Halliwell *et al.*, 1995). Lipid peroxide levels in tissue were found to be significantly increased in CCl₄-treated animals. This toxic effect is the consequence of CCl₄ activation by cytochrome P450 to trichloromethyl radical (CCl₃*) which readily reacts with oxygen to form trichloromethyl peroxy (CCl₃O₂*) radical (Tappel, 1973). The actions of these free radical triggers cell damage through two mechanisms. 1. Covalent binding to cellular macromolecules. 2. Lipid peroxidation, which affect the ionic permeability of the membrane preventing the disintegration and solubilization of membrane structure. The level of LPO decreased after treatment of MET to CCl₄-treated animals, may be attributed to the antioxidant activity of the capitula of *C. recutita* by scavenging the CCl₃* radical generated due to the metabolic transformation animals (Kalpowitz *et al.*, 1986). The SOD activity was brought to near normal after treatment with the MET in animals treated with CCl₄. Presumably, a decrease in CAT activity could be attributed to cross-linking and inactivation of the enzyme protein in the lipid peroxides. Decreased CAT activity in CCl₄ treated animals may be due to linkup to exhaustion of the enzyme because of oxidative stress caused by CCl₄. The CAT activity was restored to normal after treatment with extract evidently shows the antioxidant property of the MET against oxygen free radicals. Therefore, a significant increase in SOD and CAT activities and decrease in LPO indicates the antilipid peroxidation and/or adaptive nature of the system as brought by the MET against the damaging effect of free radical produced by the CCl₄. Recently, it has been reported that *Thespesia populnea* bark extracts, AET and Met, at a dose level of 500 mg kg⁻¹ showed significant antioxidant activity against CCl₄-induced liver injury in rats (Ilavarasan *et al.*, 2003). The mode of action of the extract of *Nymphaea stellata* wild, flower in affording the hepatoprotective against CCl₄-induced toxicity may be due to the cell membrane stabilization, hepatic cell regeneration and activation of antioxidative enzymes such as glutathione peroxidase, superoxide dismutase and catalase (Bhandarkar and Khan, 2004).

In conclusion, present results demonstrate that MET of *C. recutita* has definite antioxidant effect. The mode of action of MET in affording the hepatoprotective activity against CCl₄ may be due to the cell membrane stabilization, hepatic cell regeneration and activation of antioxidant enzymes such as GRD, GPX, GST, SOD and CAT. Although the precise mechanism of action of MET has not been elucidated, it can be assumed that the lowering of enzyme levels and increased level of LPO is responsible for cell injury and increasing the level of enzyme activities responsible for antioxidant activity. Free radical reactions are generally responsible for the progression of cancer, inflammation,

arteriosclerosis, hepatocellular damage and the biological process of ageing. Therefore, the hepatoprotective action combined with antioxidant activity has a synergistic affect to prevent the process of initiation and progress of hepatocellular diseases (Wilkinson, 1962).

Acknowledgements

Financial assistance of the DBT, New Delhi, is gratefully acknowledged. The authors are thankful to Dr. Aseem Chauhan, Chairman and Director General K.K. Ohari, Amity University, Lucknow Campus, Lucknow for their support and encouragement.

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