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ANTIOXIDANT ACTIVITY OF *CURCULIGO ORCHIOIDES* IN CARBON TETRACHLORIDE-INDUCED HEPATOPATHY IN RATS

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ABSTRACT

In this study antioxidant activity of methanol extract of rhizomes of *Curculigo orchioides* (MEC) was investigated using carbon tetrachloride (CCl_4)-intoxicated rat liver as the experimental model. The hepatotoxic rats were administered MEC for 90 days (daily, orally at the dose of 70 mg per kg body weight). Lipid peroxidation (LPO) in CCl_4 - intoxicated rats was evidenced by a marked increment in the levels of thiobarbituric acid reactive substances (TBARS) and diene conjugates (CD), and also a distinct diminution in glutathione (GSH) content in the liver. In CCl_4 + MEC – treated rats these biochemical parameters attained an almost normal level. The decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GRD) in CCl_4–intoxicated rats, and its retrieval towards near normalcy in CCl_4 + MEC- administered rats revealed the efficacy of MEC in combating oxidative stress due to hepatic damage. Elevated level of glutathione transferase(GTS) observed in hepatotoxic rats too showed signs of returning towards normalcy in MEC co-administered animals, thus corroborating the antioxidant efficacy of MEC. The findings provide a rationale for further studies on isolation of active principles and its pharmacological evaluation.

KEY WORDS

Antioxidant enzymes, Carbon tetrachloride , *Curculigo orchioides*, Lipid peroxidation.

INTRODUCTION

Curculigo orchioides Gaertn. of Amaryllidaceae family is a herbaceous tuberous geophilous perennial with rootstock bearing several fleshy lateral roots (rhizomes). It is widely distributed in India. The rhizomes of this plant possess medicinal properties and are sweet, cooling, diuretic, aphrodisiac, viriligenic and tonic which can be used against hemorrhoids, leucorrhoea, pruritis, skin diseases, asthma, bronchitis and jaundice etc. (1) Curculigo saponin G, isolated from rhizomes of the plant has been reported to increase weight of thymus gland *in vivo* in mice. (2) Ethanolic extract of *C.orchioides* has been reported to have sedative, anticonvulsant and androgen-like effect, and also adaptive effects, such as enhancing tolerance towards hypoxia and hyperthermia (3). Our previous study (unpublished data) revealed hepatoprotective potential of methanol extract of *C. orchioides*. Therefore in the present work attempt has been made to study the antioxidant effect of *C.orchioides* in CCl_4-intoxicated experimental rats.

It has been hypothesized that one of the principal causes of CCl_4–induced liver injury is LPO by free radical derivatives of CCl_4. Thus the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 - induced hepatopathy. (4)

Antioxidant action has been reported to play a crucial role in the hepatoprotective capacity of many plants, such as *Curcuma longa, Ganoderma formosanum, Solanum nigrum, Boehmeria nivea and Spirulina maxima*. (5-9) Ayurveda, an indigenous system of medicine in India, has a long tradition of treating liver disorders, with plant drugs. Thus search for crude drugs of plant origin with antioxidant activity has become a central focus of study of

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hepatoprotection. This may prove effective in alleviating tissue damages prevalent in organisms as a consequence of exposure to toxins of extrinsic or intrinsic origin.

MATERIALS AND METHODS

Plant Material

Rhizomes of *C.orchioides* were collected from Thattekkad, Ernakulam district of Kerala . The materials were identified and authenticated by experts in the Post Graduate and Research Department of Botany, St. Thomas college, Pala, Kottayam. The collected materials were thoroughly washed in water, chopped, air dried at 35 – 40 °C for a week and pulverized in electric grinder. The powder obtained was successively extracted in petroleum ether (60-80 °C), benzene, chloroform and methanol by using Soxhlet extractor. The methanol extract was then made to powder with the help of rotary evaporator under reduced pressure. Rhizomes of *C. orchioides* yielded 2.6% w/w of powdered methanol extract (MEC) which was stored in refrigerator for further use. LD 50 of MEC was found to be 180 mg/kg body weight of animals.

Experimental Animals

Twenty four male albino rats of Sprague- Dawley strain weighing 100-120 g were purchased from Small Animals' Breeding Centre of Kerala Agricultural University, Mannuthy, Trichur. The animals were housed in polypropylene cages maintained in controlled temperature (27 ± 2°C) and light cycle (12h light and 12 h dark). They were fed with Amrut Laboratory Animal feed manufactured by Nav Maharashtra Chakan Oil Mills Ltd. Pune. Food and water were provided *ad libitum*. The animals were given a week's time to get acclimatized with the laboratory conditions.

CCl_4 – induced liver damage

Hepatopathy was induced in animals by subcutaneous (sc) administration of CCl_4 at lower abdomen twice a week at the dose of 1 ml per kg. body weight in double the volume of liquid paraffin (Ip) which served as a vehicle. CCl_4 was administered on the first and fourth day of every week.

Experimental Procedure.

Body weight of animals was recorded and then they were divided into 3 groups of 8 rats each. Group – I animals served as control, which received sc administration of Ip only twice a week at the dose of 3ml per kg body weight of each animal. Group – II constituted the hepatotoxic group which received sc administration of CCl_4 + Ip twice a week as mentioned elsewhere. Group III were the herb-treated ones which received sc administration of CCl_4 + Ip twice a week as mentioned above. They also received MEC daily at the dose of 70 mg/kg body weight (effective dose) of each rat in a suspension of 1 ml water, orally by intubation. A pilot study revealed that MEC evoked hepatoprotection at doses ranging 40-120 mg/kg body weight of animals. (Figure 1) Animals were maintained at laboratory conditions for a period of 90 days.

Animals were fasted overnight on the 89th day. On the next day, after recording body weight, the animals were sacrificed by decapitation and blood was collected by the incision of jugular vein. The liver was dissected out, blotted off blood, rinsed in phosphate buffered saline (pH 7.4) and immediately proceeded for biochemical estimations. Serum was prepared from the collected blood.

Biochemical Estimations

The measurement of thiobarbituric acid reactive substances (TBARS) was done as an index of LPO. (10) CD content was found out by the method of Klein (11). Activities of SOD and CAT were determined by the methods of Marklund and Marklund, (12) and Aebi.H, (13), respectively. GSH content was determined after deproteinisation by the method of Beautler and Kelly, (14). GPX was assayed by the method of Rotruck et al., (15). Glutathione transferase (GTS) and GRD were assayed by the methods of Habig et al., (16), and Racker (17), respectively.

Statistical Analysis

The results were presented as the mean ± SEM. Student's 't' test was used to analyse statistical significance.

RESULTS

The concentration of TBARS and CD was significantly higher in liver of CCl_4-treated rats, as compared to normal control animals. (Table 1). These constituents were found to attain a near

normal level in liver of CCl_4 +MEC – treated rats. Conversely, GSH content in liver of Group – II animals showed a significant decline when compared with controls. But in Group III animals GSH content was found to attain near normalcy.

Activities of antioxidant enzymes are presented in Table 2. The levels of SOD, CAT, GPX, and GRD recorded a significant decline in CCl_4- administered rats, when compared with normal controls. In CCl_4 +MEC- treated rats, the activities of these enzymes attained a near-normalcy. However, the activity of GTS was significantly higher in CCl_4 – treated animals, which was brought down towards normalcy in herb-treated rats.

DISCUSSION

Ample experimental and epidemiological studies support the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases. (18) It is now known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). The high reactivity of ROS may trigger a host of disorders in body resulting in tissue damage and necrosis in many instances. (19)

CCl_4 – mediated hepatotoxicity was taken here as the experimental model for liver injury. It has been established that CCl_4 is accumulated in hepatic parenchymal cells and metabolically activated by cytochrome P-450 dependent monoxygenases to form a trichloromethyl free radical ($CCl_3^{\cdot}$) which alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides leading to liver damage. (20)

A study using methanol extract of *C.orchioides* rhizomes having doses ranging 40 - 120 mg/ kg body weight revealed the extract with dose 70 mg/ kg body weight offering the maximum hepatoprotection with respect to different liver marker enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT). [Refer Figure I].

The body has an effective mechanism to prevent and neutralize the free radical – induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, CAT, GPX and GRD etc. When the balance between ROS production and antioxidant defences is lost, 'oxidative stress' results, which through a series of events deregulates the cellular functions leading to various pathological conditions. (21) Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

In the present study, elevated level of TBARS and CD observed in CCl_4- treated rats indicates excessive formation of free radicals and activation of LPO system resulting in hepatic damage. TBARS produced as byproducts of LPO that occurs in hydrophobic core of bio-membranes. (22) The significant decline in the concentration of these constituents in the liver tissue of CCl_4 +MEC-administered rats indicates anti-lipid peroxidative effect of *C.orchioides.*

GSH is a major non-protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defence processes. Perturbation of GSH status of a biological system has been reported to lead to serious consequences. (21). Decline in GSH content in the liver of CCl_4- intoxicated rats, and its subsequent return towards near- normalcy in CCl_4 +MEC- treated rats reveal antioxidant effect of C. orchioides. Explanations of the possible mechanism underlying the hepatoprotective properties of drugs include the prevention of GSH depletion and destruction of free radicals. (23) These two factors are believed to attribute to the hepatoprotective properties of C. orchioides.

SOD, CAT and GPX constitute a mutually supportive team of defence against ROS. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence by lowering the steady-state level of $O_2^{\cdot}$ CAT is a hemeprotein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 and $OH^{\cdot}$. GPX is a seleno-enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In our study, decline in the activities of these enzymes in CCl_4- administered rats revealed that LPO and oxidative stress elicited by CCl_4 – intoxication have been nullified due to the effect of *C.orchioides.* This observation perfectly agrees with

those of Lin *et al.*, (8) who investigated hepatoprotective and antioxidant activity of *Boehmeria nivea*.

GTS plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. GRD is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidised glutathione to reduced form. The activities of these enzymes were found to be in the reverse order. In liver tissues of CCl_4- administered rats, level of GTS registered a significant increment, whereas that of GRD recorded a decline. However, these enzymes restored an almost normal activity in CCl_4 + MEC – administered rats, thus unearthing the antioxidant effect of *C. orchioides*.

Natural antioxidants strengthen the endogenous antioxidant defences from ROS ravage and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In conclusion, it can be said that methanol extract of rhizomes of C. *orchioides* exhibit a liver protective effect against CCl_4- induced hepatotoxicity and possessed anti-lipid peroxidative and antioxidant activities. Efforts are in progress here to isolate and purify the active principle involved in the hepatoprotective efficacy of this medicinal plant.

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Table 1

Effect of *Curculigo orchioides* on the antioxidant status of liver in rats

Parameters	Group I	Group II	Group III
Thiobarbituric acid reactive substances - TBARS (μ mol / mg protein)	0.81 ± 0.05	1.08 ± 0.04*	0.86 ± 0.04**
Diene conjugates - CD (μ mol / 100 g tissue)	0.24 ± 0.03	0.72 ± 0.06*	0.28 ± 0.05**
Reduced glutathione – GSH (μ mol / 100 g tissue)	378.6 ± 17.6	206.4 ± 10.8*	349.2 ± 11.9**

Values are mean ± SEM of 8 animals in each group.

* $P < 0.01$ as compared to Group I.

** $P < 0.01$ as compared to Group II.

Table 2

Effect of *Curculigo orchioides* on activity of antioxidant enzymes

Parameters	Group I	Group II	Group III
Superoxide dismutase - SOD (U / mg protein)	12.63 ± 0.31	7.69 ± 0.38*	11.93 ± 0.59**
Catalase - CAT (U / mg protein)	8.38 ± 0.39	4.86 ± 0.32*	7.93 ± 0.41**
Glutathione peroxidase - GPX (U / mg protein)	0.82 ± 0.09	0.59 ± 0.07 *	0.80 ± 0.09**
Glutathione reductase - GRD (U / mg protein)	6.94 ± 0.52	2.99 ± 0.32*	5.99 ± 0.44**
Glutathione transferase -GTS (μ mol / mg protein)	8.28 ± 0.82	15.61 ± 0.94*	8.19 ± 0.61**

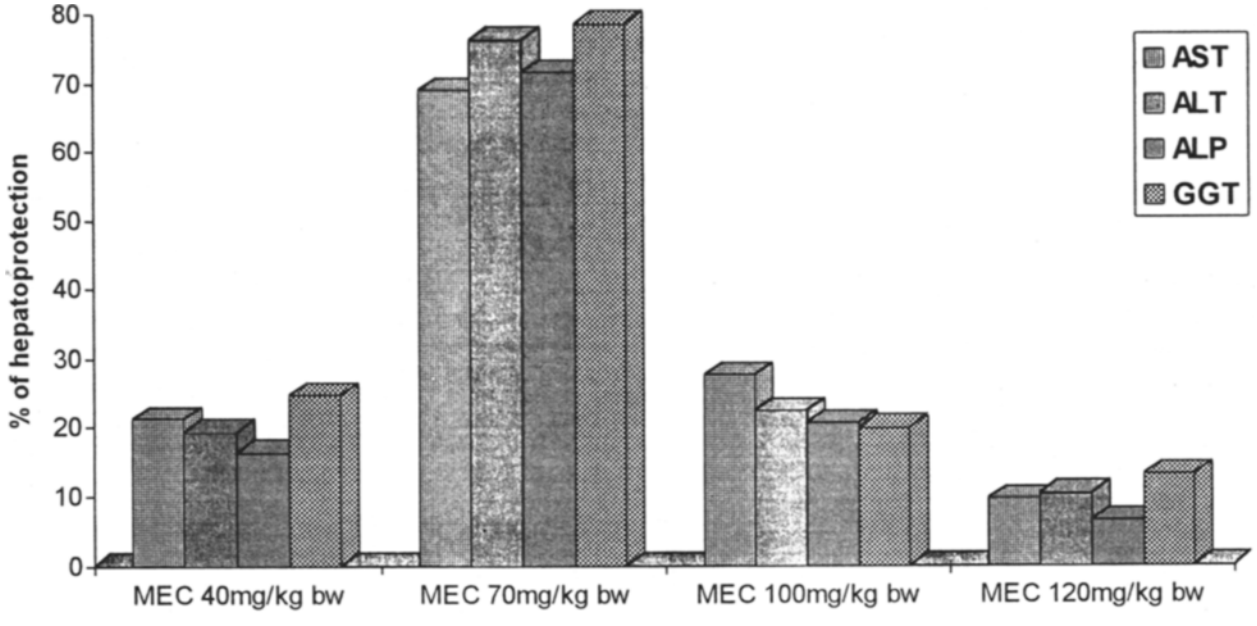
Values are mean ± SEM of 8 animals in each group

* $p < 0.01$ as compared to Group I.

** $p < 0.01$ as compared to Group II.

Figure 1

Percentage of hepatoprotection offered by methanol extract of rhizomes of *Curculigo orchioides* in respect of liver marker enzymes



AST – Aspartate aminotransferase ALT – Alanine aminotransferase ALP – Alkaline phosphatase

GGT – Gamma glutamyl transpeptidase MEC – Methanol extract of rhizomes of *C. orchioides*