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Evaluation of Hepatoprotective activity of Methanol extract of *Curculigo Orchioides* in CCl_4-Induced Liver Injury in rats

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ABSTRACT

The present study investigated the hepatoprotective activity of methanolic rhizome extract of *Curculigo orchioides* (MECO) in CCl_4-induced hepatotoxicity model in rats. The hepatoprotective activity of methanolic rhizome extract of *Curculigo orchioides* were evaluated against CCl_4-induced hepatic damage in rats. The three doses of MECO (100, 200 and 400 mg/kg) were administered orally once daily for seven days. Serum glutamate oxaloacetate transaminase (AST), serum glutamate pyruvate transaminase (ALT), serum alkaline phosphatase (ALP) and total bilirubin were estimated along with the estimation of superoxide dismutase (SOD) and malondialdehyde (MDA) levels in liver tissues. Further histopathological examination of the liver sections was carried out to support the induction of hepatotoxicity and hepatoprotective efficacy. The extract revealed significant activities and substantially elevated serum enzymatic levels of AST, ALT, ALP and total bilirubin were found to be normalized significantly by the MECO in a dose dependent manner with maximum hepatoprotection observed at 400 mg/kg dose level. The histopathological observations also indicated the biochemical evidences of hepatoprotection. Elevated level of superoxide dismutase (SOD) and decreased level of malondialdehyde (MDA) further affirmed the hepatoprotective observations. The results of the present study demonstrated that MECO have potent hepatoprotective activity against CCl_4-induced hepatic damage in experimental animals.

Keywords: hepatoprotective, antioxidant, carbon tetrachloride, *Curculigo orchioides*, Silymarin

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INTRODUCTION

Curculigo orchioides Gaertn. is a traditional Ayurvedic medicinal plant belonging to the family *Amaryllidaceae; Hypoxidaceae.* The plant is widely available in Sub-tropical Himalayas from Kumaon eastwards; Western Ghats from Konkan Southwards. This plant is known as Taalmuuli, Taalpatri, Krishna Mushali, Bhuumitaala in Ayurveda. It is also named as Musli Siyaah (Unani) and Nilappanan kizhangu (Siddha/Tamil)**.** The plant is traditionally Nervine, adaptogenic, sedative, anticonvulsive, androgenic, anti-inflammatory and diuretic. It is used in Jaundice, urinary disorders, skin diseases and asthma. The plant is Mucilaginous and the rhizome contains saponins (curculigo saponin C and F promoted proliferation of spleen lymphocytes very significantly; F and G increased the weight of the thymus *in vitro* in mice); sapogenins; phenolic glycosides, a triterpene alcohol; a pentacyclic triterpene, an aliphatic compound, hentriacontanol, sitosterol, stigmasterol, cycloartenol and sucrose. A peptide, Curculin C, containing 114 amino acids, has been isolated from the fruit. In traditional Chinese medicine, dried rhizome, containing curculigoside is used as a tonic for its immunological and protective property. In Indian medicine, powdered rhizomes with milk are taken as a restorative tonic, also for sexual debility. EtOH (50%) of the plant exhibited hypoglycaemic property[1,2].

The liver detoxifies the body by regulating the synthesis, secretion and metabolism of xenobiotics. Various physiochemical functions of the body including oxidation, reduction, hydroxylation, hydrolysis, conjugation, sulfation, acetylation etc. are co-ordinated by the liver alone. Injury to liver and damage to the hepatic parenchyma are always proved to be associated with distortion of different metabolic functions of liver [3]. Etiologically various infectious agents like viruses and different hepatotoxic chemicals including environmental pollutants are responsible for different type of liver damage and hepatic injury. Recent research also revealed the pathophysiological role of free radicals and oxidative stress in liver damage and injury. Proper understanding the mechanism of actions of potent hepatotoxins such as CCl_4, paracetamol etc. also sugested the role of oxidative stress and free radicals in the pathophysiology of hepatic injury ad damage[4]. The free radicals are normally generated during the normal body metabolic pathways and contain mostly unpaired electrons. The oxygen radicals, such as superoxide radical (O_2^-), hydroxyl radical ($^{\bullet}OH$) and non free radical species, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^{\bullet}O_2$), are generated in many redox processes of normal physiochemical pathways[5]. Antioxidant defense system comprising different enzymes such as superoxide dismutase, catalase and glutathione peroxidise etc. trap and destroy these free radicals. Vitamin

deficiency together with overproduction of free radicals and a reduced level of above mentioned enzymes, is considered as the main culprit for producing oxidative stress[6].

The most common experimental inducer of hepatotoxicity in animals is CCl_4 [7]. As the modern system of medicine did not come up with a cure to hepatic damage or injury, here started the exploration of traditional systems of medicine including Ayurveda, Siddha, Unani etc. for a probable answer to hepatotoxicity[8] Numerous medicinal plants are being researched for an effective hepatoprotective remedy.

A number of medicinal preparations in the Indian system of medicine (Ayurveda) have been used as effective hepatoprotective. In view of this several medicinal preparations and a number of medicinal plants mentioned in Ayurveda for treatment of liver disorders are being investigated [9]. Moreover traditional folklore and indigenous knowledge of medicinal uses of plants are also now being explored and documented for possible bioactive molecules to be future drugs. Therefore in this present study an attempt has been made to evaluate the hepatoprotective activity of *Curculigo orchioides* rhizome extract with a view towards elucidating the probable mechanism of action.

MATERIALS AND METHODS

Chemistry

Malondialdehyde (MDA) was obtained from Sigma Chemicals Company, St Louis, MO, USA. Silymarin was obtained from Ranbaxy Laboratories, Delhi, India. CCl_4 was obtained from E Merck, Mumbai, India. All other reagents and chemicals used in the experiments were of analytical grade and available commercially via reputed vendors.

Preparation of plant extract

The rhizomes of the plant material were collected from barkot village, Dehradun, Uttarakhand and authenticated by Dr. R.L.Painuli, Department of Botany,HNB Garhwal University, Srinagar Garhwal, Uttarakhand. The rhizomes of *Curculigo orchioides* were dried in shade and coarsely powdered. Coarsely powdered rhizomes (5 kg) was successively extracted in the Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate, methanol and water as solvent for the complete extraction of the phytochemicals. The five extracts were dried in rotary evaporator at 45 °C and the dried extracts were stored in vacuum desiccators containing anhydrous silica gel. All the five extracts were subjected to acute toxicity studies as per the OECD guidelines.

Acute toxicity studies

An acute oral toxicity study was done according to the OECD guidelines for the testing of chemicals, Test No. 423 (OECD, 2001; Acute oral toxicity-Acute toxic class method). Wistar

rats (n = 3) of either sex were selected by a random sampling technique for the acute toxicity study. The animals were fasted overnight prior to the experiment and maintained under standard laboratory conditions. Each extract was administrated orally in increasing dose up to 2000 mg/kg.

***In vivo* hepatoprotective activity**

Test Animals

Wistar rats (180–240 g) of either sex were used for the study. The animals were kept in large, clean polypropylene animal cages in a temperature-controlled room (22 ± 2 ◦C with relative humidity (44–55%) under 12-h light and dark cycles. All the animals were kept for one week prior to experiments for acclimatization to the laboratory environment. Animals were provided with a standard rodent pellet diet and clean drinking water *ad libitum*. The care and use of laboratory animals were strictly in accordance with the guidelines prescribed by the Institutional Animal Ethical Committee constituted under the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Experimental design

A total of 36 rats were divided into 6 groups of 6 rats each. Group I served as normal control and received only the vehicle (1 ml/kg/day of 1% CMC; p.o.). Group II received CCl_4 1 ml/kg (1:1 of CCl4 in olive oil) i.p. once daily for 7 days. Group III received CCl_4 1 ml/kg (1:1 of CCl4 in olive oil) i.p. and silymarin 100 mg/kg orally (p.o.) for 7 days. Groups IV, V, VI were administered MECO at 100, 200, and 400 mg/kg body weight p.o. respectively and dose of 1 ml/kg i.p. of CCl_4 (1:1 of CCl_4 in olive oil) for 7 days. All rats were sacrificed by cervical dislocation 24 h after the last scheduled treatment. Blood was collected from the retro-orbital sinus plexus under mild ether anesthesia, just before sacrifice. Collected blood was allowed to clot and serum was separated at 3500 rpm for 15 min for carrying out further biochemical investigations. One part of liver was dissected out and used for biochemical and histopathological studies.

Measurement of serum biochemical parameters

The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin were determined using the Hitachi 912 clinical chemistry automatic analyzer (Roche Diagnostic GmbH, Mannheim, Germany).

Assessment of lipid peroxidation and superoxide dismutase (SOD)

In chilled normal saline excised livers were perfused to remove all the blood cells. Then they were cut down into small pieces, placed in 0.1M phosphate buffer (pH 7.4), and homogenized

using remi homogenizer to obtain 20% homogenate. The homogenate thus obtained was centrifuged at 3000 rpm for 15 min and the supernatant was collected in an Eppendorf tube. This supernatant was again centrifuged at 12,000 rpm for 30 min. The final supernatant was used for the determination of malonaldehyde (MDA) as a lipid peroxidation marker[10]. Superoxide dismutase (SOD) was also assayed by the method described previously [11]

Histopathology

The liver tissue was dissected out and fixed in 10% formalin solution. It was then dehydrated in ethanol (50-100%), cleared in xylene and embedded in paraffin wax. Afterwards thick sections (5–6 mm) were made and then stained with hematoxylin and eosin dye for photomicroscopic observation. Scoring on scale of 1-4 was done for the liver sections under microscope as given below[12,13].

- 0 = Normal liver histology.
- 1 = Tiny and short septa of connective tissue without influence on the structure of hepatic lobules.
- 2 = Large septa of connective tissue, flowing together and penetrating into the parenchyma. Tendency to develop nodules.
- 3 = Nodular transformation of the liver architecture with loss of structure of hepatic lobules.
- 4 = Excessive formation and deposition of connective tissue with subdivision of the regenerating lobules and with development of scars.

Statistical analysis

The data were expressed as mean ± SD. Statistical differences at $p < 0.001$ between the groups were analyzed by one-way ANOVA followed by Turkey as *post hoc* using GraphPad Instat software package.

RESULTS AND DISCUSSION

Acute toxicity studies

All the extracts of *Curculigo orchioides* rhizomes did not found to cause any mortality upto 2000 mg/kg dose level. Hence 1/20 th, 1/10th and 1/5 th of the maximum dose (i.e., 100, 200 and 400 mg/kg, p.o.) were selected for the present study.

***In vivo* hepatoprotective activity**

Effect of MECO on the measurement of serum biochemical parameters

The serum biochemical parameters due to the hepatoprotective effects of MECO in CCl_4-

intoxicated rats are shown in **table 1**. CCl_4 treated rats (Group II) demonstrated a significant increase in serum AST, ALT, ALP and total bilirubin levels as compared to control group animals (Group I). MECO Pre-treatment (100, 200 and 400 mg/kg) for 7 days (Groups IV, V and VI) demonstrated significant hepatoprotection as evident from the serum AST, ALT, ALP and total bilirubin levels as compared to the toxic control group animals (Group II). Pre-treatment with the standard hepatoprotective agent-Silymarin (Groups III) also demonstrated normalized biochemical parameters.

Effect of MECO on MDA and SOD levels

Elevated MDA levels in the toxic control group demonstrated increased lipid peroxidation, when compared with the normal control group. Pre-treatment with MECO at 100, 200 and 400 mg/kg significantly decreased the MDA levels, which were almost similar to those of rats receiving the standard drug Silymarin. The level of antioxidant enzyme, SOD was found to be significantly increased in animal group treated with MECO. The extract at the highest studied dose of 400 mg/kg indicated maximum hepatoprotection (table 1).

Histopathology

With the appearance of vacuolated hepatocytes and degenerated nuclei the rats treated with CCl_4 demonstrated abnormal liver architecture as suggested by histopathological observations (Figure 1). In the central lobular area, vacuolization, fatty changes and necrosis of hepatocytes were found to be severe. CCl_4 intoxication led to excessive formation of deposition of connective tissue and development of scars (score of 4). MECO at 100 mg/kg dose did show significant hepatoprotective activity. Liver sections of rats treated with MECO (100 mg/kg) revealed nodular transformation of liver architecture with loss of structure of hepatic lobules (score of 3). The liver sections of rats treated with MECO 200 mg/kg demonstrated penetration of large septa of connective tissue flowing together into the parenchyma (score of 2). Liver sections of rats treated with MECO 400 mg/kg revealed more or less normal lobular pattern with short septa of connective tissue and a mild degree of fatty change, and necrosis (score of 1) which is almost comparable to the control animal group and silymarin treated animal gro.

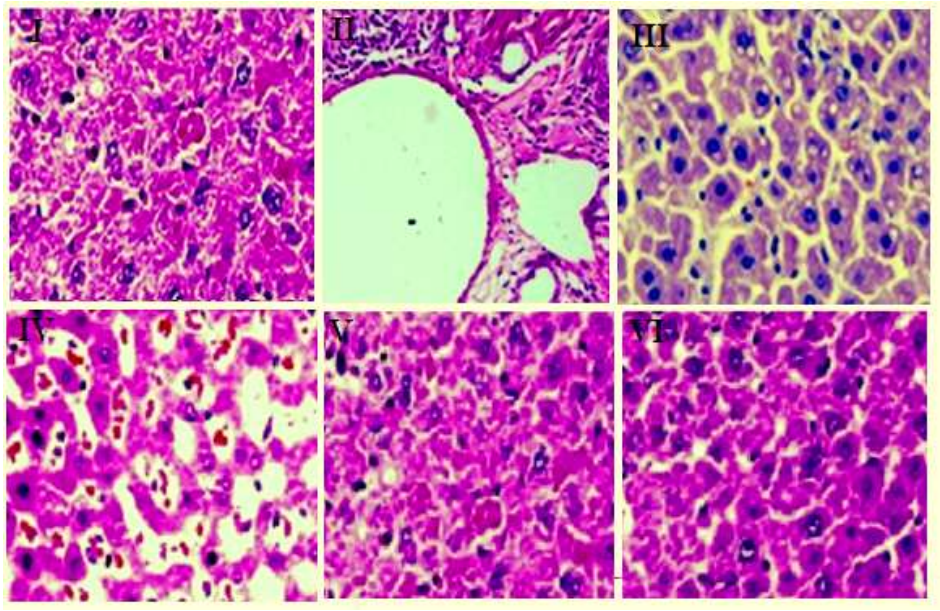

Figure 1. Representative photomicrographs of liver sections from Group I to Group VI.

Table 1. Effects of MECO on serum biochemical parameters in CCl_4-intoxicated rats.

Group	Treatment	AST (SGOT) (IU/L)	ALT (SGPT) (IU/L)	ALP (IU/L)	Serum Billirubin (mg/dl)	MDA	SOD
Group I	Control	170 ± 2.3[a]	55 ± 4.5[a]	190 ± 3.4[a]	0.40 ± 0.04[a]	90 ± 0.01[a]	8.0 ± 0.05[a]
Group II	CCl_4 1 ml/kg (i.p.)	610 ± 28.5	121 ± 8.1	541 ± 6.7	0.87 ± 0.04	120 ± 1.3	3.2 ± 0.08
Group III	Silymarin 100 mg/kg + CCl_4 (prophylactic)	230 ± 13***	61 ± 1.7***	190 ± 2.8***	0.32 ± 0.05***	101 ± 1.5***	8.0 ± 0.06***
Group IV	MECO 100 mg/kg + CCl_4 (prophylactic)	422 ± 3.4***	76 ± 3.4***	253 ± 4.9***	0.60 ± 0.06***	123 ± 2.2***	5.3 ± 0.23***
Group V	MECO 200 mg/kg + CCl_4 (prophylactic)	312 ± 18***	70 ± 2.3***	207 ± 4.1***	0.62 ± 0.09***	111 ± 0.56***	5.7 ± 0.13***
Group VI	MECO 400 mg/kg + CCl_4 (prophylactic)	242 ± 4.7***	58 ± 3.4***	202 ± 6.0***	0.50 ± 0.11***	121 ± 0.67***	7.8 ± 0.17***

MECO: Methanolic extract of *Curculigo orchoides*. Data are expressed as mean ± SD (n = 6). One-way ANOVA Tukey *post hoc*:***p<0.001

The present study established the hepatoprotective effects of MECO against CCl_4–induced liver injury in rats. By detoxifying the chemicals and xenobiotics, the liver becomes the main target organ for all possible toxicities caused by xenobiotics. CCl_4 is a potent hepatotoxin, and is the, most extensively used chemical agent to investigate hepatoprotective activity on various experimental animal models. The experimental hepatic damage caused by CCl_4 histologically also resembles viral hepatitis [14]. CCl_4 is biotransformed in liver by cytochrome P_{450} enzymes to a very active radical, CCl_3 radical. This active CCl_3 radical readily reacts with oxygen to produce trichloromethylperoxyl radical ($CCl_3O_2^{\cdot}$), which is then covalently binds with cellular macromolecules and biomembranes to cause lipid peroxidation of the lipid membranes of the adipose tissue, leading to bio-membrane damage. Peroxide products of lipid peroxidation finally initiate production and leakage of biomarkers like MDA (Malonaldehyde). This whole cascade of biochemical events finally causes loss of hepato-cellular integrity and hepatic damage [15]. Lipid peroxidation is very important parameter of oxidative stress along with other free radical damage, which occurred in the above mentioned biochemical cascade. Therefore, antioxidant efficacy is known as a hallmark parameter indicative of possible mechanism of hepatoprotection. Normally, liver contains AST, ALT and ALP as the serum hepatobillary enzymes in high concentrations. When there is necrosis or hepatic damage, these enzymes will be leaked into the circulation; raising serum concentration of these enzymes as detected during hepatic damage [16]. Elevated serum AST, ALT and ALP levels in CCl_4 treated animals indicated cellular breakage and loss of functional integrity of cell membranes in liver [3, 16]. In the present study, CCl_4 (Group II animals) induced hepatic damage as indicated by increased MDA levels in liver due to increased lipid peroxidation. Reduced estimation of SOD in CCl_4 treated animals also suggested failure of antioxidant defense mechanism to block peroxidation damage. In view of this, the increased serum level of AST, ALT and ALP enzymes in CCl_4 treated animals (Group II) confirmed hepatic damage. As a breakdown product of heme in red blood cells, bilirubin is regarded as a clinical and pathophysiological indicator of necrosis of liver tissues. MECO Pretreatment in different animal groups (Group IV/V/VI) resulted a significant decrease in serum AST, ALT, ALP and total bilirubin levels as compared to CCl_4 treated group (Group II). Prophylactic use of MECO resulted in an inhibition of the degree of hepatic necrosis and concomitantly decreased the leakage of intracellular enzymes by stabilizing hepatic cellular membranes. The results are further confirmed by the histopathological observations. Inhibition of lipid peroxidation to a significant degree is also a predominant mechanism of hepatoprotection as suggested by the significant decrease in MDA levels. Increase in the SOD level was also

suggestive of repairment of antioxidant defense system, which plays an important role in hepatoprotection. Based upon the results of this present study, it can be concluded that the methanol rhizome extract of *Curculigo orchioides* has proven itself as a significant hepatoprotective.

CONCLUSION

The present study clearly demonstrated the 'in vivo' effectiveness of the extract in terms of lipid peroxidation inhibitory capacity and further confirmed the significant hepatoprotective activity of the methanol extract of rhizomes of *Curculigo orchioides*.

REFERENCES

1. Anonymous. Ayurvedic Pharmacopoeia of India. In: Ayush, editor. 1 ed. New Delhi: Ayush; 2004.
2. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary: Springer; 2007.
3. Wolf PL. Biochemical diagnosis of liver diseases. Indian Journal of Clinical Biochemistry 1999;14:59-90.
4. Oh TY, Lee JS, Ahn BO, Cho H, Kim WB, Kim YB, et al. Oxidative damages are critical in pathogenesis of reflux esophagitis: implication of antioxidants in its treatment. Free Radical Biology and Medicine 2001;30:905-15.
5. Gülçin I, Oktay M, Küfrevioglu ÖI, Aslan A. Determination of antioxidant activity of lichen Cetraria islandica (L) Ach. J Ethnopharmacology 2002;79(3):325-9.
6. Ellnain-Wojtaszek M, Kruczynski Z, Kasprzak J. Investigation of the free radical scavenging activity of Ginkgo biloba L. leaves. Fitoterapia 2003;74(1-2):1-6.
7. Johnston DE, Kroening C. Mechanism of early carbon tetra chloride toxicity in cultured rat hepatocytes.;:. Pharmacol Toxicol 1998;83:231-9.
8. Subramoniam A, Pushpangadan P. Development of phytomedicines for liver disease. Indian J Pharmacol 1999;31:166-75.
9. Chatterjee TK. Herbal Options. 1 st ed. Kolkata: Books and Allied (P) Ltd; 2000.
10. Nourooz-Zadeh J, Tajaddini-Sarmadi J, McCarthy S, Betteridge DJ, Wolff SP. Elevated levels of authentic plasma hydroperoxides in NIDDM. Diabetes 1995;44:1054-8.
11. Misra HP, Fridovich I. Role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 1972;247:3170.
12. Hirayama C, Morotami I, Hiroshige K. Quantitative and metabolic changes of hepatic collagens in rats after carbon tetrachloride poisoning. Biochemical Journal 1979;118:229-

32.

13. Ala-Kokko L, Stenback F, Ryhanen L. Preventive effect of malotilate on carbon tetrachloride induced liver damage and collagen accumulation in the rat. Biochemical Journal 1987;246:503-9.
14. James GWL, Pickering RW. The protective effect of a novel compound RU-18492 on galactosamine induced hepaptotoxicity in rats. Drug Research 1976;26:2197-9.
15. Thabrew MI, Joice PDTM, Rajatissa WA. Comparative study of the efficacy of Paetta indica and Osbeckia octandra in the treatment of liver dysfunction. Planta Medica 1987;53:239-41.
16. Drotman R, Lawhan G. Serum enzymes are indications of chemical induced liver damage. Drug Chem Toxicol 1978;1:163–71.

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