

Potential Hepatoprotective Effect and Antioxidant Role of Methanol Extract of *Oldenlandia umbellata* in Carbon Tetrachloride Induced Hepatotoxicity in Wistar Rats

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ABSTRACT

The present work examines the protective mechanisms of methanol extract of *Oldenlandia umbellata* (MEOU) in carbon tetrachloride intoxicated rats. Rats are treated with carbon tetrachloride at the dose of 1 ml/kg body weight intraperitoneally once every 72 hrs for 16 days. The hepatoprotective activity of methanol extract of *Oldenlandia umbellata* was evaluated by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP). The serum levels of total protein and bilirubin were also estimated. The histological studies were also carried out to support the above parameters. Administration of MEOU (250 and 500 mg/kg, p.o.) significantly ($p < 0.05$) prevented CCl_4 -induced elevation of levels of serum GPT, GOT, ALP, and bilirubin. The total protein level was decreased due to hepatic damage induced by CCl_4 and it was found to be increased in methanol extract of *Oldenlandia umbellata* treated group. Treatment of rats with CCl_4 led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA). This was associated with a significant reduction of the hepatic antioxidant system e.g. reduced glutathione (GSH) and Catalase. These biochemical alterations resulting from CCl_4 administration were significantly ($p < 0.05$) inhibited by pretreatment with methanol extract of *Oldenlandia umbellata*. The results are comparable with standard drug silymarin. A comparative histopathological study of liver exhibited almost normal architecture, as compared to CCl_4 treated control group. These data suggest that the methanol extract of *Oldenlandia umbellata* may act as a hepatoprotective and antioxidant agent.

Keywords: *Oldenlandia umbellata*, Hepatoprotective, Carbon tetrachloride, Antioxidants

Liver is the main organ responsible for drug metabolism and appears to be sensitive target site for substances modulating biotransformation [1]. During the course of aerobic metabolic reactions, considerable amounts of Reactive Oxygen Species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are generated [2], which undergo a variety of chain reactions and produce free radicals such as OH^* . These hydrogen species attack polyunsaturated fatty acids and thereby initiate the process of lipid peroxidation [3] resulting in degradation and inactivation of various important biomolecules.

Herbs have recently attracted attention as health beneficial foods and as source materials for drug development. Herbal medicines derived from plant extracts

are being increasingly utilized to treat a wide variety of clinical diseases including liver disease [4], ischemia, reperfusion injury, atherosclerosis, acute hypertension, haemorrhagic shock, diabetes mellitus and cancer [5] with relatively little knowledge regarding their modes of action [6].

Various species of *Oldenlandia* are used medicinally in Indian and Chinese traditional medicine. *Oldenlandia umbellata* Lamk, (synonyms: *Hedyotis hispida*, *Hedyotis indica*) (family: Rubiaceae) which is commonly known as "Imbural", is naturalized throughout the sea-coasts region of Tamilnadu, Orissa and West Bengal in India. In folklore medicine this plant is widely used in the treatment of various ailments. The decoction of the plant is widely used as an expectorant and febrifuge. It

is also used in treatment of cancer, asthma and tuberculosis [7,8]. Literature survey of this medicinal revealed that no extensive phytochemical and pharmacological investigations had been carried out.

Keeping the above information in view, the present study was designed to induce CCl₄ hepatotoxicity and demonstrate the protective role of *Oldenlandia umbellata* in rats.

MATERIALS AND METHODS

Plant material

The aerial parts of the plant *Oldenlandia umbellata* were collected from Lalgudi, Tamilnadu, India. The plant material was identified by Dr. (Mrs.) Banumathi, Department of Botany, St Joseph College, Trichy, Tamilnadu, India and the voucher specimen (GMT-1) has been preserved in our research laboratory for future reference. The aerial parts of the plant were dried under shade and powdered with a mechanical grinder. The powdered plant material was then passed through sieve # 40 and stored in an airtight container for future use.

Extraction of the powdered plant material

The air-dried powdered plant material (2kg) was defatted with Petroleum ether in a Soxhlet extraction apparatus. The defatted plant marc was successively extracted with methanol. The solvents were completely removed under reduced pressure to obtain a dry mass. The yields of the petroleum ether and methanol extracts were found to be 8.6 and 13.5 % w/w respectively. The extracts were stored in a vacuum desiccator and subjected to various chemical tests to detect the presence of different phytoconstituents. A weighed amount was suspended in 0.025% Carboxyl methyl cellulose (CMC) prior to administration.

Animals

Adult male Wistar albino rats weighing 150–180 g were used for the present investigation. They were housed in clean polypropylene cages and were fed with standard pellet diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 h. All procedures were approved by Jadavpur University Animal Ethical Committee, Kolkata, India.

Chemicals and Drugs used

Silymarin was purchased from Micro labs (Hosur, Tamilnadu, India), 1-Chloro-2,4-dinitrobenzene [CDNB], bovine serum albumin (Sigma chemical St. Louis, MO, USA), thiobarbituric acid, nitrobluetetrazolium chloride (NBT) (Loba Chemie, Mumbai, India), 5,5'-dithio bis-2-nitrobenzoic acid (DTNB), carbon tetrachloride, (SICCO research laboratory, Mumbai). All

other chemicals and solvent were of analytical grade commercially available.

Experimental protocol

Adult male Wistar albino rats weighing 150–180g were used for the experiment. The following experimental protocol was followed for the present investigation [9]. The animals were divided into five groups (n=6). Animals in group one served as a vehicle control, which received 0.025 % carboxymethylcellulose solution (CMC). Group two to five were treated with CCl₄ in liquid paraffin (1:2) at the dose of 1 ml/kg body weight intraperitoneally once in every 72 h for 16 days. Methanol extract of *Oldenlandia umbellata* (MEOU) at the doses of 250 mg/kg and 500 mg/kg, b.w. were administered orally to the animals in group three and four for 16 days. Group five received silymarin as a Standard drug at the dose of 25 mg/kg, b.w. orally for 16 days.

All the animals were sacrificed after collecting the blood from retro-orbital plexus under ether anaesthesia for biochemical estimations. The blood samples were allowed to clot and the serum was separated by centrifugation at 37°C and used for the assay of biochemical marker enzymes. Liver tissues were collected for biochemical and histopathological examination.

Biochemical estimations

Serum glutamic oxaloacetic and glutamic pyruvic transaminase activities [10] and alkaline phosphatase [11] were determined. The total protein concentration and bilirubin were measured by the method of Lowry et al [12] and Oser [13].

Lipid Peroxidation

Liver tissue was collected from each experimental rat, homogenized in 1.15% KCl and centrifuged at 1200 rpm at 4°C for 10 min. The supernatant was collected and was again centrifuged at 10,000 rpm at 4°C for 10 minutes. Again the supernatant was collected and centrifuged at 54,000 rpm for 60 minutes at 4°C.

From this microsomal fraction, lipid peroxidation in liver was ascertained by the formation of malondialdehyde (MDA) and measured by the thiobarbituric acid-reactive substance method according to Ohkawa et al [14]. The levels of lipid peroxides were expressed as 'n' moles of thiobarbituric acid substances (TBARS)/mg protein using extinction co-efficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Glutathione (GSH)

For measure GSH, tissue fragments was thawed and homogenized on ice in 1 ml of homogenizing buffer (250 mM sucrose, 20 mM Tris-HCl, 1mM dithiothreitol, pH 7.4), using glass-Teflon homogenizers. The homogenates were centrifuged at 75,000 rpm at 4°C for 2 h. The reduced glutathione was determined by the method of Ellman et al [15].

Table 1. Effect of Meou And Silymarin on Serum Biochemical Parameters in Ccl4 Intoxicated Rats

Biochemical parameters	Normal animals	CCl ₄ Treated	CCl ₄ + MEOU (250 mg/kg)	CCl ₄ + MEOU (500 mg/kg)	CCl ₄ + Silymarin (25 mg/kg)
SGOT (IU/L)	70.08 ± 1.26	158.2 ± 0.52	128.03 ± 0.2*	88.3 ± 0.73*	80.32 ± 0.7
SGPT (IU/L)	62.27 ± 0.52	145.6 ± 0.89	110.96 ± 0.73*	78.3 ± 0.62*	64.56 ± 0.2
ALP (IU/L)	20.7 ± 0.2	70.4 ± 0.9	45.7 ± 0.1*	35.4 ± 0.8*	28.4 ± 0.6
Total bilirubin (mg/dl)	0.8 ± 0.001	4.3 ± 0.08	2.8 ± 0.03*	1.8 ± 0.01*	1.3 ± 0.07
Total protein (mg/dl)	7.06 ± 0.07	3.60 ± 0.11	4.38 ± 0.07*	5.42 ± 0.08*	6.03 ± 0.07

All values represent the mean ± SEM. P values calculated by ANOVA followed by Dunnett's post hoc test of significance. $p < 0.05$ implies (Each value represents the mean ±SEM, n=6 in each group)

Catalase activity (CAT)

Liver tissue was homogenized in M/150 phosphate buffers in ice and centrifuged at 2000 rpm for 10 minutes at 4°C. From the supernatant, Catalase activity was assayed by the method of Aebi [16].

Histological observation

After sacrificing the rats by cervical dislocation, liver tissue were collected, washed in normal saline and fixed by using fixative (picric acid, formaldehyde 40% and Glacial acetic acid) for 24 h and dehydrated with alcohol. Liver tissues were cleaned and embedded in paraffin (melting point 58-60°C), cut in 3-5µm sections, stained with the haematoxylin-eosin dye and finally, observed under a photomicroscope and morphological changes such as cell necrosis, ballooning degeneration, fatty changes or inflammation of lymphocytes were observed [17].

Statistical analysis

Experimental results were expressed as mean ± S.D. Analysis of variance was performed by ANOVA procedures (SSPS 9.0 for Windows). Significant differences between means were determined by Dunnett's post hoc test. $p < 0.05$ implies statistically significance.

RESULTS

The effect of methanol extract of *Oldenlandia umbellata* on serum transaminases, alkaline phosphatase, bilirubin and total protein levels in CCl₄ intoxicated rats are summarized in Table 1. There was a significant increase in serum GPT, GOT, ALP and bilirubin levels. The total protein levels were significantly decreased in CCl₄ intoxicated rats. The methanol extract of *Oldenlandia umbellata* at the doses of 250 and 500 mg/kg orally significantly ($p < 0.05$) decreased the elevated serum marker enzymes and reversed the altered total protein to almost normal.

The effect of MEOU on lipid peroxidation (expressed in terms Thiobarbituric acid reactive substances (TBARS)) glutathione levels and catalase levels in rat liver tissue are shown in Fig 1. Lipid peroxidation levels were significantly increased and glutathione levels were

significantly decreased in CCl₄ treated rats when compared with that of the normal animals. Treatment with methanol extract of *Oldenlandia umbellata* at the doses of 250 and 500 mg/kg orally significantly decreased ($p < 0.05$) the elevated lipid peroxide levels and restored the altered glutathione levels in CCl₄ intoxicated rats. The MEOU at the doses of 250 and 500 mg/kg restored the altered catalase level in CCl₄ intoxicated rats.

Histological observation of liver tissue of the normal animal showed hepatic cells with well-preserved cytoplasm, nucleus, nucleolus, and central vein. In CCl₄ treated group, histological observation showed fatty degeneration, damage of parenchymal cells, steatosis and hydropic degeneration of liver tissue. Prominent damage of central lobular region appeared in the liver. The MEOU restored the histopathological abnormality induced by CCl₄.

DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxins used in the experimental study of liver diseases [18,19,20]. It was found that chronic administration of CCl₄ produced liver cirrhosis in rats. It is well documented that carbon tetrachloride is biotransformed under the action of cytochrome p 450 - 2e1 (CYP2e11) in the microsomal compartment of liver to trichloromethyl (*CCl₃) and peroxytrichloromethyl (Cl₃COO*) free radical [21,22,23]. These free radicals bind covalently to the macromolecules and induce peroxidative degradation of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides followed by pathological changes such as

- Triglyceryl accumulation due to blockage in synthesis of lipoprotein
- Polyribosomal disaggregation
- Depression of protein synthesis
- Elevated levels of serum marker enzyme such as SGOT, SGPT and ALP
- Depletion of glutathione
- Increased lipid per oxidation
- Cell membrane break down and death [24]

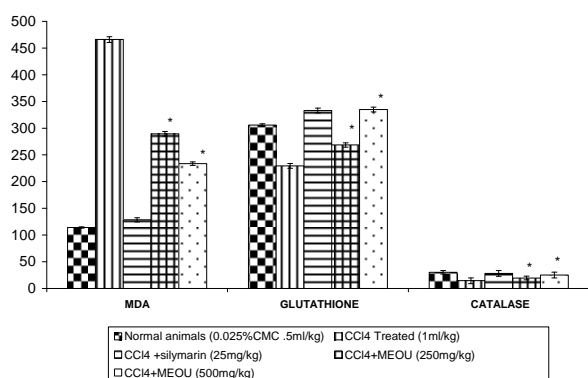


Fig 1. Effect of Meou on Lipid Peroxidation and Biomarker Enzymes in Carbon Tetrachloride Intoxicated Rats.

The elevated levels of serum enzymes are indicative of cellular leakages and loss of functional integrity of cell membrane in liver. Serum ALP and bilirubin levels on the other hand are related to the function of hepatic cells [25]. MEOU has significantly decreased the serum GOT, GPT towards normal level. These indicate that MEOU preserved the structural integrity of the hepatocellular membrane and liver cell architecture damage caused by CCl₄, which is confirmed by histopathological studies.

CCl₄ intoxication also produced significant rise in serum bilirubin thereby indicating hepatic damage [26]. It is well known that necrotizing agents like CCl₄ produce sufficient injury to hepatic parenchyma to cause elevation in bilirubin content in plasma [26,27]. These effects induced by CCl₄, were confirmed by our results. The MEOU at the doses of 250 and 500 mg/kg orally for sixteen days significantly restored the altered ALP and total bilirubin levels. CCl₄ intoxication significantly lowered total protein levels and at 250 and 500 mg/kg MEOU orally significantly increased protein levels and also preserve the structural integrity of the hepatocellular membrane and liver cell architecture damaged by CCl₄, which was confirmed by histopathological studies.

Lipid peroxidation has been postulated to be the destructive process of liver damage due of CCl₄ intoxication [22]. Lipid peroxide levels were significantly increased in CCl₄ intoxicated rats. The increase in MDA (in terms of TBARS) suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals [28,29]. CCl₄ intoxicated animals treated with 250 and 500 mg/kg, b.w. MEOU orally for sixteen days, had significantly reduced lipid peroxide levels in compared with rats treated with CCl₄ only.

Glutathione (GSH) is one of the abundant tripeptide non-enzymatic biological antioxidants present in the liver [30,31]. It acts as a substrate for the H₂O₂ removing enzyme glutathione peroxidase and for dehydroascorbate reductase [32]. In addition GSH plays a critical role in cellular function which includes the maintenance of membrane protein, the removal of free

oxygen radicals such as peroxy radical, super oxide radical, alkoxy radical, translocation of amino acids across cell membranes, the detoxification of foreign compounds and biotransformation of drugs [33,34].

CCl₄ intoxication produced significant depletion of GSH and imbalance of GSH/GSSG ratio. The reduced form of GSH becomes readily oxidized to GSSG on interacting with free radicals [35]. Excessive production of free radicals resulted in the oxidative stress, which leads to damage to biomolecules e.g. lipids, and can induce lipid peroxidation *in-vivo* [36]. In our present study the decrease level of GSH was associated with enhanced lipid peroxidation in CCl₄ intoxicated rats. Administration of MEOU restored the depleted glutathione levels in a dose dependant manner. Thus MEOU may act by inducing the detoxifying enzymes and these enzymes might detoxify the free radicals produced following carbon tetrachloride intoxication.

Catalase is an enzymatic antioxidant widely distributed in all animal tissues [37]. Catalase decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [38]. Therefore reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide [39]. Administration of MEOU restored the activities of catalase in CCl₄ intoxicated rats. Our results revealed that MEOU prevented excessive free radicals accumulation and protected the liver from CCl₄ intoxication.

Our phytochemical study showed the presences of flavanoids, steroids, terpenoids, and tannins in MEOU. It is known that some flavanoids are able to reduce xenobiotic-induced hepatotoxicity in animals [40,41]. The inhibitory activity of flavanoids on free radical production could be related their hepatoprotective effects since exogenous antioxidants may counteract the damaging effects of oxidative stress, cooperating with natural systems like glutathione, tocopherol or protective enzymes [42].

In summary, the results of this study demonstrate that methanol extract of *Oldenlandia umbellata* has a potent hepatoprotective action on CCl₄ induced hepatic damage in rats. These results show that the hepatoprotective effects of MEOU may be due to its ability to block the bioactivation of CCl₄ by inhibiting P450-2e1 and improving the structural integrity of the hepatocyte and its antioxidant activity, in combination with its ability to scavenge free radicals and inhibit lipid peroxidation, all of which are capable of hepatocellular injury.

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