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POTENTIAL HEPATOPROTECTIVE EFFECT AND ANTIOXIDANT ROLE OF METHANOL EXTRACT OF *MORINDA TINCTORIA* IN CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

The present work examines the protective mechanisms of methanol extract of *Morinda tinctoria* in carbon tetrachloride intoxicated rats. Rats are treated with carbon tetrachloride at the dose of 1 ml/kg body weight intraperitonially once every 72 hrs for 14 days. The hepatoprotective activity of methanol extract of *Morinda tinctoria* 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 extract (400and 800 mg/kg) significantly ($p< 0.05$) prevented CCl4-induced elevation of levels of serum GPT, GOT, ALP, and bilirubin. 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 *Morinda tinctoria* may act as a hepatoprotective and antioxidant agent.

Keywords: *Morinda tinctoria* **,** Hepatoprotective, Carbon tetrachloride, Antioxidant.

INTRODUCTION

Liver is the main organ responsible for drug metabolism and appears to be sensitive target site for substances modulating biotransformation. During the course of aerobic metabolic reactions, considerable amounts of Reactive Oxygen Species (ROS) such as superoxide anion (O2-) and hydrogen peroxide (H_2O_2) are generated , 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 resulting in degradation and inactivation of various important biomolecules [4, 20, 22]. 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, ischemia, reperfusion injury, atherosclerosis, acute hypertension, haemorrhagic shock, diabetes mellitus and cancer with relatively little knowledge regarding their modes of action[1, 9, 26] .Various species of *Morinda tinctoria* are used medicinally in Indian traditional medicine. *Morinda tinctoria* (synonyms: *Morinda pubescens, Morinda tomentosa, Morinda coriea*) (family: Rubiaceae) which is commonly known as “Nunaa”, is naturalized throughout parts of TamilNadu and in some parts of Kerala,inIndia. In Indian traditional system of medicine, leaves and roots of Morinda tinctoria are used as astringent, deobsterent, emmengogue, to relieve pain in the gout, as tonic, in liver diseases and febrifuge. It is also used for curing dyspepsia, diarrhoea, ulceration, stomatitis, digestion, wound and fever. The leaf juice is useful as a local application. Root is used to cure inflammation and boils. Unripe fruit is used to cure rheumatism. Ash of the fruit prevents dysentery, vomiting, diarrhoea and cholera.There is greater demand for fruit extract of Morinda species in the treatment of

arthritis, cancer, gastric ulcer and other heart disease. Literature survey of this medicinal revealed that no extensive photochemical 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 *Morinda tinctoria* in rats.

MATERIALS AND METHODS

Plant material: The aerial parts of the plant *Morinda tinctoria* [1, 26] were collected from paripally,kerala,India. The plant material was identified by Dr. Boby, Department of Botany, TKM college,Kollam, Kerala, India and the voucher specimen has been preserved in our research laboratory for future reference (UCP/MGU/RIMSR/2011/herb16).The stem bark 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 (1kg) was defatted with Ethyl acetate 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 ethyl acetate and methanol extracts were found to be 4 and 4.5 % 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 rats weighing 150–200 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 Animal Ethical Committee, IAEC NO: IAEC/UCP, MGU RIMSR/ 1067/2011.

Acute toxicity study: This was performed for the extracts to ascertain safe dose by the acute oral toxic class method by the Organization of Economic Cooperation and Development (OECD). The methanolic Extract of *Morinda tinctoria* was studied for acute toxicity at dose of 2000 mg/kg i.p. in rats.

Chemicals and Drugs used: Silymarin was purchased from sami labs (Bengaluru India) and all other chemicals and solvent were of analytical grade and commercially available.

Biochemical estimations: The biochemical parameters like SGOT, SGPT, ALP and Bilirubin were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit using Autoanalyser.

In vitro Antioxidant studies [17, 19, 20]

DPPH radical scavenging activity: The free radical scavenging capacity of the extracts was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. Methanol extract of *M.tinctoria was* mixed with 95% methanol to prepare the stock solution (5 mg/mL). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and *M.tinctoria* extracts was added followed by serial dilutions (100 µg to 500 µg) to every test tube so that the final volume was 3 mL and after 10 min, the absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (100 µg to 500 µg). Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank.

Hepatoprotective activity [5, 6, 7, 9]

Method of inducing liver damage: Liver damage can be induced by the administration of certain toxic chemicals like carbon tetrachloride, chloroform, ethanol, cadmium chloride and various hydrocarbons. The excessive use of certain drugs like paracetamol, tetracycline, methyl dopa etc. can also produce liver damage. In majority of the studies, the well known hepatotoxin Carbon tetrachloride (CCl_4) has been used to induce experimental liver damage. Hence in this study also CCl_4 has been selected to produce hepatocellular damage.

Methodology [12, 13, 14]: The albino rats were divided into six groups, each group had six animals. Group I (control) animals were administered a single daily dose of carboxy methyl cellulose (1 mL of 1%, w/v, p.o. body weight). Group II received carbon tetrachloride (1 mL/kg body weight, i.p. 1:1 v/v mixture of CCI_4 and liquid paraffin) alone while group III, IV, V received orally 200, 400, 800 mg/kg body weight of MEMT in (1 %, w/v, CMC) respectively along with carbon tetrachloride as in

group II. Group VI received silymarin, the known hepatoprotective compound (Sami Labs, Banglore, India), at a dose of 100 mg/kg, p.o. along with carbon tetrachloride. The extract of plant was given daily while carbon tetrachloride was given for every 72 h for 14 days. All the animals were sacrificed after collecting the blood from retro-orbital plexus under ether anesthesia 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.

Statistical analysis: Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance followed by Tukey-Kramer Multiple Comparisons Test. P value less than <0.05 was considered to be statistically significant.*P<0.05, **<0.01 and ***<0.001, when compared with control and toxicant group as applicable.

RESULTS

Rats subjected to the CCl_4 challenge alone developed liver injury as evident from the elevation in the biochemical markers, like SGPT, SGOT, ALP, Bilirubin Oral administration of the test extract exhibited dose dependent significant reduction in the CCl4 induced change in the biochemical levels. Treatment with the reference standard, silymarin (100 mg/kg p.o.) also reversed the hepatotoxicity significantly. Hepatoprotective potency of the methanolic extract at the dose 800 was found closer to that of standard.

Determination of Biochemical parameters.*:* Effect of Methanolic and ethyl acetate extracts of *Morinda tinctoria*. and Silymarin on enzyme AST (SGOT), ALT (SGPT), ALP, Bilirubin levels in blood serum of CCl4 induced liver damage is given in table 1.

Histopathological Studies in CCl4 induced hepatotoxicity: The histopathological evaluation of CCl_4 toxicity in all the groups was examined and shown in figure 1-6. The description is as follows,
Section of rat liver treated with vehicle control group (Figure. 1) shows liver parenchyma with intact architecture which is the normal appearance. Histopathological profile of liver from CCl_4 intoxicated rats (Figure. 2) showed hepatic globular architecture disrupted, hepatic cells has shown various degree of fatty degeneration, infiltration of lymphocytes and proliferation of kupffer cells. Section of liver in silymarin treated group (Figure. 6) shows liver parenchyma with intact architecture. Some of the central veins show congestion with diffuse congestion of sinusoids. Section of liver in test drug treated groups (800 mg/kg) shows intact architecture, few regenerative hepatocytes, sinusoidal congestion which is similar to silymarin treated group.

Antioxidant activity (Table:2): The DPPH radical scavenging activity of the MEMT at different concentrations (100-500µg/ml) was compared with Ascorbic acid at varying concentrations (100-500µg/ml). Graph:1 illustrates a significant decrease in the DPPH radical due to the scavenging ability of extracts and ascorbic acid. The methanolic extracts showed maximum activity of 81.99% and at 500µg/ml respectively, where as ascorbic acid at the same concentration exhibited 93.18% inhibition. The IC50 values were found to be 77µg/ml, 120µg/ml and for ascorbic acid methanolic and ethyl acetate extract respectively.

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 (*CCl_3) and peroxytrichloromethyl (Cl_3COO*) free radical [21, 22]. These free radicals bind covalently to the macromolecules and induce peroxidative degradation of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids.(7,9) 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 ,Cell membrane break down and death .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. [23, 25] Methanol extract of *Morinda tinctoria* has significantly decreased the serum GOT, GPT towards normal level. These indicate that MEMT preserved the structural integrity of the hepatocellular membrane and liver cell architecture damage caused by CCl4, which is confirmed by histopathalogical studies.CCl_4 intoxication also produced significant rise in serum bilirubin thereby

indicating hepatic damage. It is well known that necrotizing agents like CCl_4 produce sufficient injury to hepatic parenchyma to cause elevation in bilirubin content in plasma. These effects induced by CCl4, were confirmed by our results. The MEMT at the doses of 400 and 800 mg/kg for fourteen days significantly restored the altered ALP and total bilirubin levels. Lipid peroxidation has been postulated to be the destructive process of liver damage due of CCl4 intoxication. Our phytochemical study showed the presences of flavanoids, steroids, terpenoids, and tannins in MEMT. It is known that some flavanoids are able to reduce xenobiotic-induced hepatotoxicity in animals. 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. In summary, the results of this study demonstrate that methanol extract of *Morinda tinctoria* has a potent hepatoprotective action on CC14 induced hepatic damage in rats. These results show that the hepatoprotective effects of MEMT may be due to its ability to block the bioactivation of CC14 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.

Table 1: Effect of Methanolic extracts of *Morinda tinctoria*. *Roxb* and Silymarin on enzyme AST (SGOT), ALT (SGPT), ALP, Bilirubin levels in blood serum of CCl4 induced liver damage.

Animal Groups	Bilirubin	SGOT	SGPT	ALT
Group 1	0.723±0.073	74.008±3.344	44.040±1.504	62.758±4.590
Group 2	2.840±0.241	322.691±7.037	224.721±5.96	158.160±8.260
Group 3	1.701±0.210***	216.625±8.973***	137.475±8.891***	103.098±8.961***
Group 4	0.913±0.073***	125.165±10.116***	78.896±2.282***	76.598±3.303***
Group 5	0.858±0.043***	119.415±9.225***	74.44±2.536***	76.31±3.728***
Group 6	0.708±0.065***	115.02±7.589***	66.511±3.465***	75.856±6.978***

Values are mean ± SEM (n=6) one way ANOVA. Where, * represents significant at p<0.05, ** represents moderately significant at p< 0.01, and *** represents highly significant at p<0.001. All values are compared with toxicant.

Table 2: **DPPH radical scavenging activity of ascorbic acid and extracts of *Morinda tinctoria* stem bark.**

CONCENTRATION	ASCORBIC ACID	% FREE RADICAL
		MEMT
100	65.00 ± 0.005	43.84± 0.005***
200	73.14± 0.005	66.22± 0.005***
300	80.06± 0.005	71.95± 0.005***
400	83.92± 0.005 79.24	79.24± 0.005***
500	93.18± 0.005 81.99	81.99± 0.005***

Graph 1: **DPPH radical scavenging activity of ascorbic acid and extracts of *Morinda tinctoria* stem bark.**

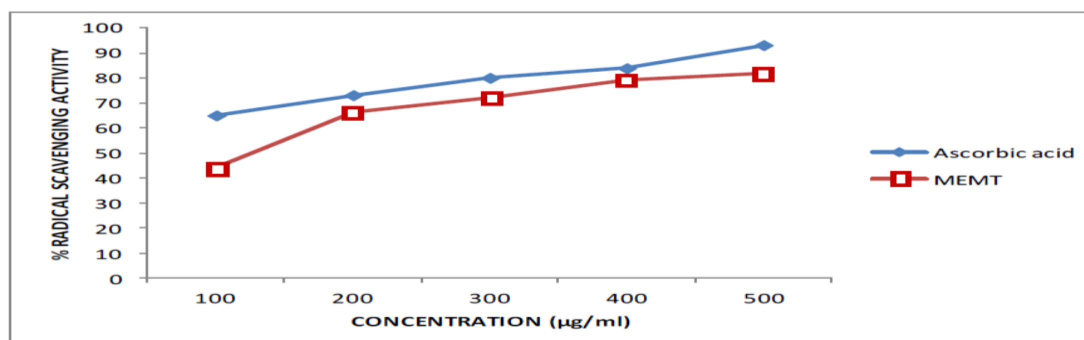

Figure 1: Control

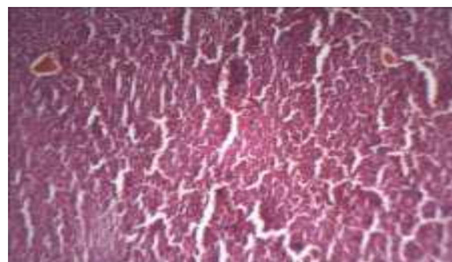

Figure 2: CCl4 Group

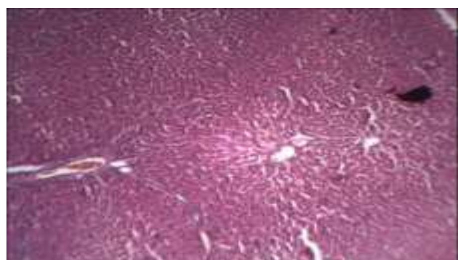

Figure 3: CCl4+MEMT 200mg/Kg

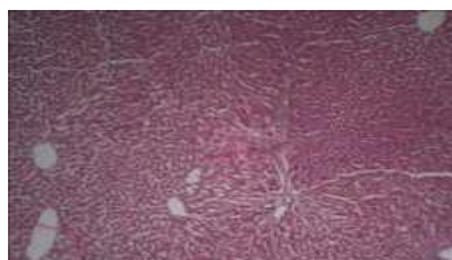

Figure 4: CCl4+MEMT 400mg/Kg

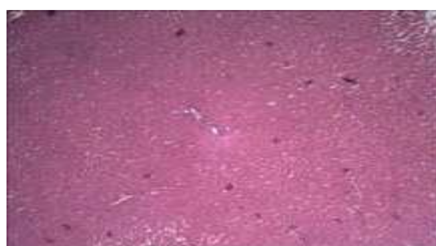

Figure 5: CCl4+MEMT 800mg/Kg

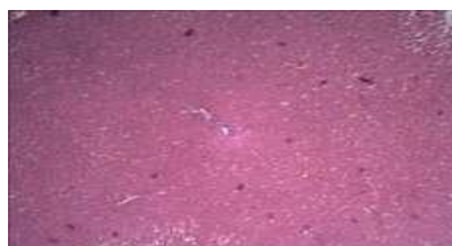

Figure 6: CCl4+Silymarin

Fig 1-6: Histo pathological studies in CCl_4 induced hepatotoxicity.

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