

## Hepatoprotective Effects of *Momordica Cymbalaria* Fenzl. against Carbon Tetrachloride Induced Hepatic Injury in Rats

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### Summary

The ethanolic extract of roots of *Momordica cymbalaria*(Cucurbitaceae) was investigated for antioxidant and hepatoprotective effects in male wistar rats.

*Momordica cymbalaria* (250, 500 mg/kg, p.o/14days) or silymarin (100 mg/kg p.o./14days) were administered to the Carbon tetrachloride (1.25ml/kg, ip/14days) treated rats. Serum AST, ALT, ALP, bilirubin, total protein, cholesterol, triglyceride and Hepatic glutathione (GSH) catalase (CAT) and super oxide dismutase (SOD) were estimated. Histopathology of the liver was also studied.

The extract or silymarin produced a significant ( $P < 0.001$ ) hepatoprotective effect by decreasing the activity of serum AST, ALT, ALP, bilirubin, total protein, cholesterol, triglyceride hepatic lipid peroxidation and increasing the levels of antioxidants markers like hepatic glutathione (GSH), catalase (CAT), and super oxide dismutase (SOD). Centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in group treated with carbontetrachloride where as the extract treated group showed the normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins without noticeable alterations.

The ethanolic extract of roots of *Momordica cymbalaria* could protect the liver cells from carbon tetrachloride induced liver damages, by its antioxidative effect on hepatocytes.

**Key words:** antioxidant, carbon tetrachloride, Hepatotoprotective, *Momordica cymbalaria*,

**Running title:** Hepatoprotection by *M. cymbalaria*

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## Introduction

Liver diseases, especially viral hepatitis occurs predominantly in the developing world with an enormous impact on public health and economy<sup>1</sup>. Carbon tetrachloride (CCl<sub>4</sub>) is widely used in animal models to induce acute liver injury<sup>2-4</sup>. It is generally believed that the toxicity of CCl<sub>4</sub> results from its reductive dehalogenation by the cytochrome P450 enzyme system into the highly reactive free radical trichloromethyl radical<sup>5</sup>. Antioxidant action has been reported to play a crucial role in the hepatoprotection<sup>6</sup>.

*Momordica cymbalaria* Fenzl (MC) (Cucurbitaceae) is a species found in the states of Karnataka and Andhra Pradesh, India. Its tuber is traditionally used as an abortifacient<sup>7</sup> and also used locally for the treatment of diabetes mellitus. We have reported its antiovarian, abortifacient activity; antiimplantation and cardio protective activities<sup>8-10</sup>. The fruit powder and extracts of MC is previously reported to have the antidiabetic activity in experimental diabetic models<sup>11-13</sup>. However its action in relation to hepatoprotection has not been reported. In the present study an attempt has been made to elucidate the effect of MC on CCl<sub>4</sub> induced hepatic damage with reference to biochemical markers, antioxidant enzymes, and histopathology.

## Materials and Method

### *Plant Material*

The fresh roots of MC were collected from Gadag district, Karnataka, identified and authenticated by Dr. Sreenath, Department of Botany, Bangalore University, Bangalore. A specimen sample of the same was preserved in the herbarium of the Department of Botany, Bangalore University, Bangalore (voucher no. 18122003). The roots of MC were isolated, chopped into small pieces, dried under shade at room temperature for seven days and powdered. The powder was extracted with ethyl alcohol to get a yield of 14.1 % w/w. Dried extract dissolved in distilled water was used for the study. Phytochemical test of the extract indicated the presence of alkaloids, saponins proteins and phytosterols.

### *Experimental animals*

Thirty male albino wistar rats weighing 100-120 g were purchased from (National Institute of Mental Health and Neuro Science) NIMHANS Bangalore. The animals were housed in polypropylene cages maintained in controlled temperature (27 ± 2°C) and light cycle (12h light and 12 h dark) and fed with standard rat pellet diet (Amrut rat and mice feed, India) and water *ad libitum*. The animals were given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC). The oral acute toxicity study was performed using the up & down procedure (OPPTS guidelines).

*Experimental Procedure*

Hepatopathy was induced in animals by administration of CCl<sub>4</sub> intraperitoneally (i.p) at the dose of 1.25ml/kg, ip, in liquid paraffin for 14 days. The rats were equally divided into 5 groups (n=6). Group I: Control, (liquid paraffin 1.25ml/kg i.p.). Group II: CCl<sub>4</sub> (1.25 ml/kg, i.p/ 14days); Group III: CCl<sub>4</sub>+ MC (250 mg/kg, p.o/14days); Group IV: CCl<sub>4</sub> and MC (500mg/kg p.o/14days); Group V: CCl<sub>4</sub>+ silymarin (100 mg/kg, p.o/14days). At the end of the treatment, rats were sacrificed by cervical dislocation, blood samples were collected by direct cardiac puncture and the serum was used for the assay of marker enzymes. Liver was dissected out and immediately washed immediately with ice-cold saline and a homogenate was prepared in 0.1 N Tris HCl buffer (pH 7.4) and the homogenate was used for the estimation of antioxidant marker enzymes.

*Serum biochemical estimations:*

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed using standard kits (SPAN India Ltd, Surat). The results were expressed as units/liter (IU/L). The levels of total protein, total bilirubin, cholesterol and triglyceride were estimated in the serum using standard commercial kits from (SPAN India Ltd, Surat, India).

*Assessment of hepatic Oxidative Stress marker enzymes:*

The liver homogenate was centrifuged at 10,000 xg at 0° for 20 minutes using Remi C-24 high speed cooling centrifuge and supernant was used for the assay of lipid peroxidation (malondialdehyde content), endogenous antioxidant enzymes, reduced glutathione (GSH) catalase (CAT), and super oxide dismutase (SOD)

*1. Estimation of lipid peroxidation*

The extent of lipid peroxidation in tissues was assessed by measuring the level of malondialdehyde (MDA) as described by Wilbur<sup>14</sup>. Briefly 1 ml of trichloroacetic acid (TCA) 20% and 2 ml of thiobarbituric acid (TBA) 0.67% were added to 2 ml of homogenate supernant. The absorbance of the mixture was recorded at 530 nm and the values were expressed as  $\eta$ M of MDA formed /mg of protein

*2. Estimation of reduced glutathione activity*

Reduced glutathione (GSH) in the rat hearts was assayed by the method described by Ellman<sup>15</sup>. Briefly 0.02ml of the homogenate supernant was added to 3ml of Ellman reagent. The changes in absorbance were read at 412  $\eta$ m. The amount of glutathione was expressed as  $\mu$ g of GSH/mg protein.

*3. Estimation of SOD activity*

The level of SOD was measured by the method of Kono<sup>16</sup>. Briefly 1.3 ml of solution A (0.1 nM EDTA containing 50 nM Na<sub>2</sub>CO<sub>3</sub>, pH 10.3), 0.5 ml of solution B (90 M NBT-nitro blue tetrazolium dye) and 0.1 ml of solution C (20mM Hydroxylamine hydrochloride, pH6.0) were mixed and the rate of NBT reduction was recorded at 560nm. SOD activity was expressed as unit/mg protein.

#### *4. Estimation of Catalase activity*

Catalase activity was estimated by determining the decomposition of H<sub>2</sub>O<sub>2</sub> at 240 nm in an assay mixture containing phosphate buffer as described by Hug O E Aebi<sup>17</sup>. The activity was expressed in units as  $\mu$ M of H<sub>2</sub>O<sub>2</sub> consumed per min/mg of protein.

*Histopathological examination:* Liver pieces were preserved in 10% formaldehyde solution for histopathological study. The pieces of liver were processed and embedded in paraffin wax. Sections of about 4-6  $\mu$ m were made and stained with hematoxylin and eosin and photographed.

**Statistical analysis:** The statistical analysis were carried out by one-way analysis of variance (ANOVA), followed by Tukey Kramer multiple comparison post-test. P values <0.05 were considered significant.

## **Results**

*Serum biochemical parameters:* The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) showed a significant (p<0.001) increase in CCl<sub>4</sub> treated rats as compared to control group (Table 1). Administering MC significantly (p<0.001) reduced the levels of AST, ALT and ALP in CCl<sub>4</sub> treated rats as compared to the animals treated with CCl<sub>4</sub> alone.

When compared to control group the total serum protein concentration was significantly (p<0.001) lower and serum total bilirubin was significantly (p<0.001) increased in CCl<sub>4</sub> treated group (Tables 1) and the levels of both significantly decreased in MC+ CCl<sub>4</sub> and Silymarin + CCl<sub>4</sub> group as compared to CCl<sub>4</sub> treated rats.

The level of triglycerides and cholesterol in serum showed a significant (p<0.001) increase in CCl<sub>4</sub> treated rats as compared to those of control group. Treatment with MC or silymarin in CCl<sub>4</sub> treated rats showed a significant decrease in triglycerides and cholesterol levels as compared to CCl<sub>4</sub> treated rats.

*Hepatic Oxidative Stress parameters:* Malondialdehyde (MDA) level was significantly (p<0.001) increased and the levels of GSH, CAT and SOD were significantly (p<0.001) decreased in CCl<sub>4</sub> treated rats when compared with those of the animals in control group. Administering MC (250 and 500 mg/kg) in CCl<sub>4</sub> administered rats significantly decreased malondialdehyde (MDA) and increased the levels of GSH, CAT and SOD (Table 2). The results are well comparable with silymarin + CCl<sub>4</sub> administered group.

Table 1 Effect of Ethanolic extract of *Momordica cymbalaria* (MC) in Carbon tetrachloride (CCl<sub>4</sub>) treated rats (1.25mg/kg, ip) on serum biochemical parameters

Groups	Treatment	AST IU/L	ALT IU/L	ALP IU/L	Total Protein gm/dl	Total Billirubin µm/L	Cholesterol mg/dl	Triglycerides mg/dl
I	Normal control	56.32 ±2.57	33.84 ±1.61	43.96 ±1.80	6.62 ±0.12	0.42 ±0.02	110.19 ±2.13	129.46 ±2.20
II	CCl <sub>4</sub>	95.76 ±4.09 <sup>†††</sup>	59.20 ±3.3 <sup>†††</sup>	106.2 ±2.97 <sup>†††</sup>	4.79 ±0.27 <sup>†</sup>	1.54 ±0.25 <sup>†††</sup>	195.8 ±3.55 <sup>†††</sup>	167.20 ±3.57 <sup>†††</sup>
III	MC 250mg/kg +CCl <sub>4</sub>	67.48 ±2.60 <sup>***</sup>	41.32 ±1.47 <sup>***</sup>	68.10 ±2.55 <sup>***</sup>	5.85 ±1.08 <sup>*</sup>	0.61 ±0.13 <sup>***</sup>	132.38 ±2.44 <sup>***</sup>	141.16 ±3.19 <sup>***</sup>
IV	MC 500mg/kg +CCl <sub>4</sub>	57.88 ±1.41 <sup>***</sup>	31.39 ±2.07 <sup>***</sup>	41.51 ±1.65 <sup>***</sup>	6.87 ±1.03 <sup>*</sup>	0.50 ±0.03 <sup>***</sup>	115.53 ±2.67 <sup>***</sup>	132.94 ±3.61 <sup>***</sup>
V	Silymarin 100mg/kg +CCl <sub>4</sub>	58.05 ±2.60 <sup>***</sup>	37.32 ±1.72 <sup>***</sup>	50.49 ±1.59 <sup>***</sup>	6.40 ±0.16 <sup>*</sup>	0.48 ±0.03 <sup>***</sup>	119.87 ±1.92 <sup>***</sup>	134.92 ±2.43 <sup>***</sup>

Values expressed as mean ±SEM, n=6; <sup>†††</sup>P<0.001, <sup>†</sup>P<0.05 considered statistically significant as compared to normal control group; <sup>\*\*\*</sup>P<0.001, <sup>\*</sup>P<0.05 considered statistically significant as compared to carbon tetrachloride treated group

Table 2 Effect of ethanolic extract *Momordica cymbalaria* (MC) on the hepatic oxidative stress parameters in Carbon tetrachloride (CCl<sub>4</sub>) (1.25mg/kg, ip) treated rats

Groups	Treatment	LPO nm of MDA / mg of protein	GSH µg /mg of protein	CAT µm H <sub>2</sub> O <sub>2</sub> /mg of protein	SOD U/mg of protein
I	Normal control	0.74 ±0.08	6.67 ±0.15	8.40 ±0.57	7.45 ±0.95
II	CCl <sub>4</sub>	1.99 ±0.26 <sup>†††</sup>	2.76 ±0.43 <sup>†††</sup>	4.91 ±0.88 <sup>†</sup>	4.73 ±1.08 <sup>†</sup>
III	MC 250mg/kg +CCl <sub>4</sub>	1.40 ±0.17 <sup>*</sup>	3.77 ±0.68 <sup>*</sup>	6.8 ±0.75 <sup>*</sup>	6.68 ±0.74 <sup>*</sup>
IV	MC 500mg/kg +CCl <sub>4</sub>	0.79 ±0.05 <sup>***</sup>	6.89 ±0.26 <sup>***</sup>	8.32 ±0.93 <sup>*</sup>	7.39 ±0.83 <sup>*</sup>
V	Silymarin 100mg/kg +CCl <sub>4</sub>	0.69 ±0.13 <sup>***</sup>	6.59 ±0.47 <sup>***</sup>	8.42 ±1.13 <sup>*</sup>	7.59 ±0.64 <sup>*</sup>

Values expressed as mean ±SEM, n=6; <sup>†††</sup> P<0.001, <sup>†</sup>P<0.05 considered statistically significant as compared to normal control group; <sup>\*\*\*</sup>P<0.001, <sup>\*</sup>P<0.05 considered statistically significant as compared to Isoproterenol control group

**Histopathological observation:** Histopathological study of liver from control group animals showed a normal hepatic architecture. (Figure 1a) In CCl<sub>4</sub> treated group, severe hepatotoxicity was evidenced by centrilobular necrosis accompanied by fatty changes and ballooning degeneration (Figure 1b). Treatment with MC or silymarin to carbon tetrachloride treated rats exhibited almost normal architecture (Figure 1c, d & e).

Fig1. Effect of ethanolic extract *Momordica cymbalaria* (MC) on the Histopathological pictures of Carbon tetrachloride (CCl<sub>4</sub>) (1.25mg/kg, ip) treated rats

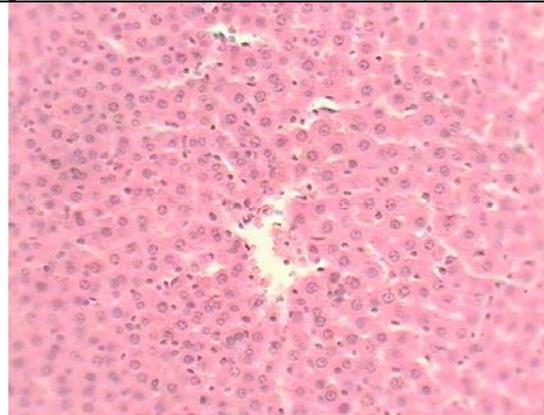


Fig 1a Group I Control

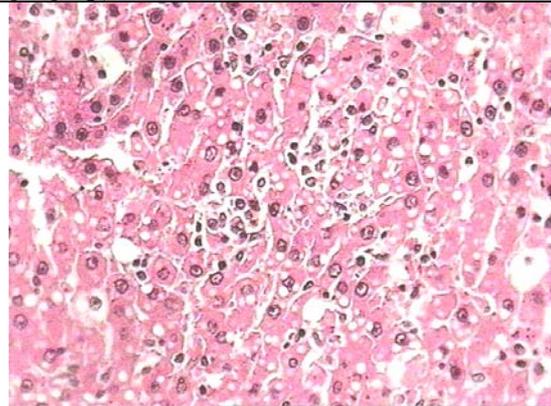


Fig 1b Group II CCl<sub>4</sub> (1.25mg/kg, ip)



Fig 1c Group III MC 250mg/kg +CCl<sub>4</sub>

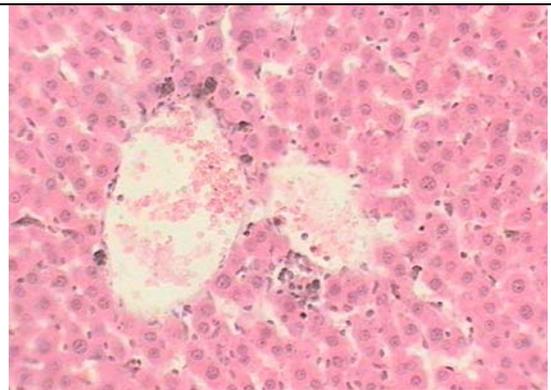


Fig 1d Group IV MC 500mg /kg +CCl<sub>4</sub>

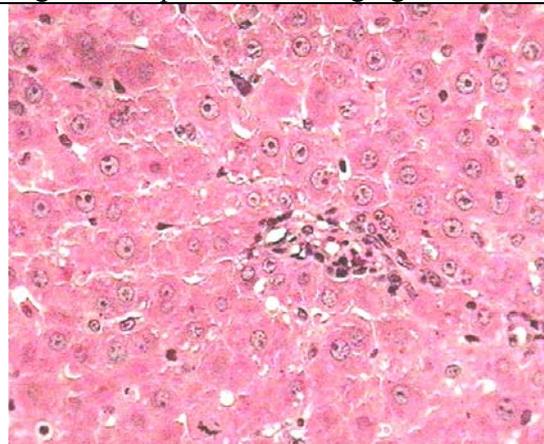


Fig 1e Group V Silymarin 100mg/kg +CCl<sub>4</sub>

### Discussion

Liver injury induced by CCl<sub>4</sub> is the best characterized system of xenobiotic-induced hepatotoxicity and is commonly used models for the screening of anti-hepatotoxic and/ or hepatoprotective activities of drugs<sup>5</sup>. The changes associated with CCl<sub>4</sub>- induced liver damage are similar to that of acute viral hepatitis<sup>18</sup>. It has been established that carbon tetrachloride accumulates in hepatic parenchymal cells and gets metabolically activated by cytochrome P-450 dependent monooxygenases form trichloromethyl free radical (CCl<sub>3</sub> •). These free radicals 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<sup>19</sup>. Lipid peroxidation will initiate pathological changes such as depression of protein synthesis<sup>20</sup>, elevation of serum marker enzymes such as AST, ALT and ALP<sup>21</sup> depletion of glutathione content, catalase activity<sup>22</sup> and further increase in lipid peroxidation<sup>23</sup>. SGPT is more specific to the liver and a better parameter for detecting liver damage<sup>24</sup>. MC at the dose of 250 and 500 mg/kg and decreased the levels of both AST and ALT significantly in CCl<sub>4</sub> treated rats indicating maintainance of functional integrity of hepatic cell membrane. Serum ALP and bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure<sup>25</sup>. In the present study MC at both the doses has been found to reduce both serum ALP and bilirubin in the treated groups compared with the untreated ones. The site specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis<sup>26</sup>. Hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases. Hence decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. The lowered level of total proteins recorded in the serum as well as liver of CCl<sub>4</sub> treated rats reveals the severity of hepatopathy. MC treated rats maintained near normalcy of total protein level. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the production of liver cells<sup>27</sup>. Treatment with CCl<sub>4</sub> increases the levels of total lipids, total triacyl glycerols and total cholesterol in liver<sup>28</sup>. Presence of significantly high concentration of total lipids and cholesterol in the serum of CCl<sub>4</sub> treated animals and maintainance of these towards near normal values in MC administered rats demonstrates the hepatoprotective effect of MC. Malondialdehyde (MDA) levels in tissue were found to be significantly elevated in CCl<sub>4</sub> treated rats. This toxic effect is the consequence of CCl<sub>4</sub> activation by cytochrome P450 to trimethyl radical (CCl<sub>3</sub> •) which readily reacts with oxygen to form trichloromethyl peroxy radicals (CCl<sub>3</sub>O<sub>2</sub>•)<sup>29</sup> (Tappel AC, 1973). These free radicals trigger cell damage through two mechanisms namely covalent binding to cellular macromolecules and lipid peroxidation which affect the ionic permeability of the membrane preventing the disintegration and solubilization of membrane structure. The diminished lipid peroxidase activity after treatment with the MC may be attributed to the antioxidant activity of the plant by scavenging the CCl<sub>3</sub> • free radical generated due to the metabolic transformation of CCl<sub>4</sub> in the liver. Glutathione content in the liver plays a primary role in the protection against trichloromethyl radical-induced liver damage.

It has been suggested that the lipid peroxidases generated after CCl<sub>4</sub> treatment is eliminated by glutathione peroxidase in the presence of glutathione, thus curbing the propagation of lipid peroxidation<sup>5</sup>. The present study found a significant decrease in hepatic glutathione, SOD and catalase levels following CCl<sub>4</sub> exposure. MC treatment significantly increased their levels which clearly indicate the effect of extract in quenching the reactive intermediates and radical species generated during oxidative stress. Histopathological studies also provided supportive evidence for the biochemical analysis. The MC treated group showed the normal parenchymal architecture without noticeable alterations compared to group II (Fig. 1a). Centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in group treated with CCl<sub>4</sub>. In conclusion, the results of this study demonstrate that ethanolic extract of *Momordica cymbalaria* Fenzl. has a potent hepatoprotective action upon CCl<sub>4</sub> induced hepatic damage in rats. Our results show that the hepatoprotective effects may be due to its antioxidant and free radical scavenging properties.

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