EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF SAPONIN OF *MOMORDICA DIOICA* ROXB. AGAINST CARBON TETRACHLORIDE INDUCED HEPATIC INJURY IN RATS

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Summary

The saponin fraction of fruits of *Momordica dioica* (Cucurbitaceae) was investigated for hepatoprotective effects in male wistar rats. The saponin fraction of *Momordica dioica* (SMD*)* (27.5 & 55 mg/kg p.o.) administered to the carbon tetrachloride treated rats. The saponin fraction or silymarin produced a significant ($P < 0.05$) hepatoprotective effect by decreasing the activity of AST, ALT, ALP, bilirubin and increasing the activity of total protein. The group treated with the SMD showed normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins without noticeable alterations. The result reveled that SMD protect the liver cells from carbon tetrachloride induced liver damages, by its antioxidative effect on hepatocytes. The drug silymarin (100mg/kg p.o) was used as standard.

Key words: Saponin, Carbon Tetrachloride, Hepatotoprotective, *Momordica dioica,* Hepatocytes, Silymarin

Introduction

Liver diseases, especially viral hepatitis occurs predominantly in the developing world with enormous impact on public health and economy [1]. Carbon tetrachloride (CCl_4) is widely used in animal models to induce acute liver injury [2, 3, 4]. It is generally believed that the toxicity of CCl_4 results from its reductive dehalogenation by the cytochrome P450 enzyme system into the highly reactive free radical trichloromethyl radical [5].

Antioxidant action has been reported to play a crucial role in the hepatoprotection [6]. *Momordica dioica* belonging to family *Curcubitaceae* found in coastal Karnataka and Andhra Pradesh state. It has been reported that the plant contain aliphatic constituents (6-methyl tritriacont-50on-28-of and 8-methyl hentracont-3-ene) and sterol (pleuchiol) and pentacylic triterpene (momodicaursenol) an unknown isolated from the seeds, has been identified as urs-12, 18(19)-dien-3 beta-ol [7].

The chloroform, ethyl acetate & ethanolic extract of *Momordica dioica roxb* fruit is previously reported to have the antidiabetic activity in alloxan induced experimental rats[8], flavanoidal fraction from ethanolic extract of the fruit is reported to have hepatoprotective property[9], hexane extract of the fruit is reported to have antifeedant property[10], seed oil has shown insecticide property[11], ethanolic and aqueous extract of the root is reported to have antifertility activity[12].

Further three triterpenes and two steroidal compounds were isolated from the dry root of *Momordica dioica* and have shown anticancer property[13]. In the present study an attempt has been made to elucidate the effect of saponin fraction of *Momordica dioica* on CCl_4 induced hepatic damage.

Materials and Methods

Plant Material

The fresh fruits of *Momordica dioica*, Roxb were collected from local market of Bangalore, Karnataka, identified and authenticated by Dr. Gajendra Rao, Survey Officer, Regional Research Institute, Bangalore. A specimen sample of the same was preserved in the herbarium section at RRI, Bangalore, as RRCBI, Acc No.1693 for future reference. The fresh fruits of *Momordica dioica* were isolated, chopped into small pieces, dried under shade at room temperature for seven days and powdered.

Extraction and Isolation of Saponins

1.5 Kg of dry powder of fruits of *Momordica dioica* was extracted with methanol and concentrated to get the dried methanolic extract. The dried methanolic extract was dissolved in hot distilled water and partitioned between water saturated n-butanol. The organic layer (n-butanolic layer) is separated and evaporated to get of residue. This n-butanolic residue was dissolved in methanol and poured diethyl ether (Et_2O) to obtain flocculent precipitate. This precipitate was separated by means of filter paper and washed with excess of diethyl ether (Et_2O) and dried to yield of crude saponin extract[14].

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_4 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_4 (1.25 ml/kg, i.p/ 14days); Group III: CCl_4+ SMD (55 mg/kg, *p.o*/14days); Group IV: CCl_4 + SMD (27 mg/kg, *p.o*/14days)**;** Group V: CCl_4+ silymarin (100 mg/kg, *p.o*/14days).
On the 8th day, 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.

Assessment of Liver Function
The levels of Aspartate AminoTransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP), Total Protein (TP) and Total Billirubin (TB) were assayed using standard kits (SPAN India Ltd, Surat). The results were expressed as units/liter (IU/L).

Histopathological Studies
For histopathological study, the livers were quickly removed after autopsy and fixed in 10% formalin. The rats were sacrificed and the livers removed were washed with normal saline. Small pieces of tissues were embedded in paraffin wax. The sections of about 5-6mcm were cut, stained and then observed under microscope for histopathological changes in liver and their pictographs were taken[15].

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.[16].

Results

The levels of serum hepatic marker enzymes AST, ALT and ALP showed a significant increase ($p<0.05$) in CCl_4 treated rats as compared to control group (Table 1). Administering SMD significantly reduced ($p<0.05$) the levels of AST, ALT, ALP in CCl_4 treated rats as compared to the animals treated with CCl_4 alone.

The total serum protein concentration was significantly lower ($p<0.05$) and serum total billirubin was significantly ($p<0.05$) higher in CCl_4 treated group (Tables 1) when compared to control group and the level of total serum protein was significantly increased and total serum billirubin was significantly decreased in CCl_4 + SMD and CCl_4 + Silymarin group when compared to CCl_4 treated rats.

Histopathological study of liver from control group animals showed a normal hepatic architecture (Figure 1a). In CCl_4 treated group, severe hepatotoxicity was evidenced by centrilobular necrosis accompanied by fatty changes and ballooning degeneration (Figure 1b). Treatment with SMD or silymarin to carbon tetrachloride treated rats exhibited almost normal architecture (Figure 1c, d & e).

Table 1: Effect of SMD serum level of asparate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP) and total bilirubin (TB) in experimental groups

Group	Treatment Design	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	TP (g/dl)	TB (g/dl)
01	Vehicle control	56.31± 1.050	33.84± 0.065	43.96± 0.730	6.62± 0.120	1.26± 0.009
02	CCl_4 treated	95.76± 4.090*	59.2± 1.300*	106.16± 1.210*	4.7± 0.031*	2.54± 0.100*
03	Saponin fraction of *Momordica dioca* (55mg/kg/day)+CCl_4	65.35± 2.401†	39.20± 0.650†	54.55± 0.920†	6.08± 0.040†	1.57± 0.016†
04	Saponin fraction of *Momordica dioca* (27.5mg/kg/day)+CCl_4	80.00± 2.010†	49.60± 0.700†	70.42± 1.110†	4.85± 0.080†	2.03± 0.033†
05	Silymarin (100mg/kg/day) + CCl_4	58.04± 2.600†	37.16± 0.640†	50.48± 0.650†	6.40± 0.040†	1.32± 0.016†

Values are mean ± SEM, n=6.

**P<0.05 when compared with control group.*

†*P<0.05 when compared with CCl_4 treated group.*

Figure 1: Histopathological changes in the liver of Wistar Rats

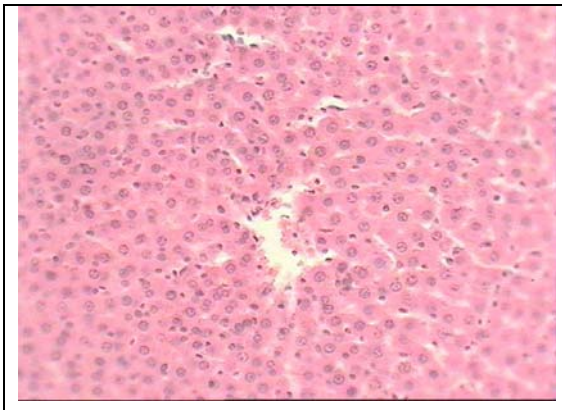

Fig 1a Group I Control

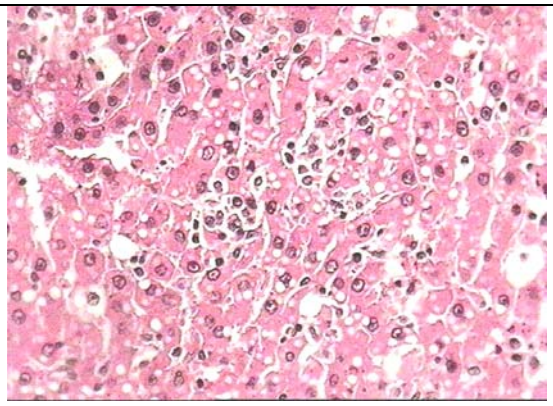

Fig 1b Group II CCl_4 (1.25mg/kg, ip)

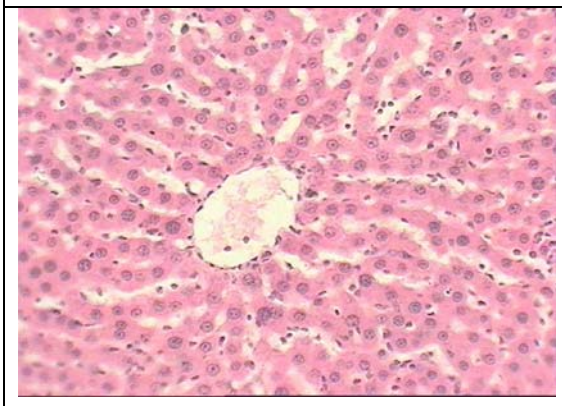

Fig 1c Group III SMD 55mg/kg +CCl_4

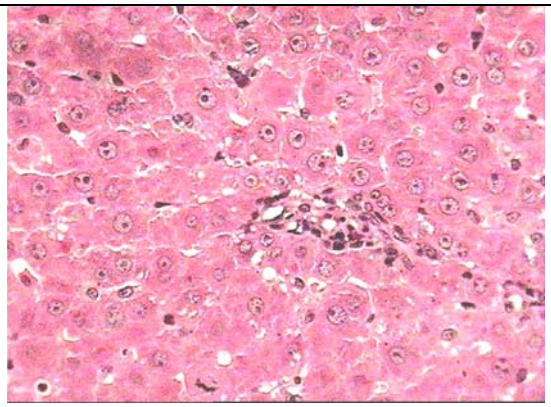

Fig 1d Group IV SMD 27.5mg /kg +CCl_4

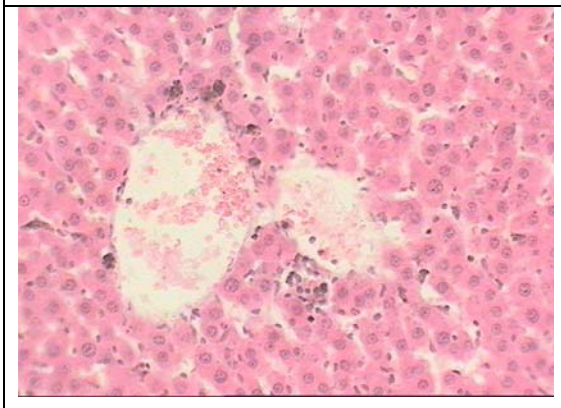

Fig 1e Group V Silymarin 10mg/kg +CCl_4

Discussion

It has been established that carbon tetrachloride accumulates in hepatic parenchymal cells and gets metabolically activated by cytochrome P-450 dependent monoxygenases form trichloromethyl free radical (CCl_3 •). These free radicals alkylate 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[17].

Lipid peroxidation will initiate pathological changes such as depression of protein synthesis[18], elevation of serum marker enzymes such as AST, ALT and ALP. The SMD at the dose of 27.5 and 55 mg/kg decreased the levels of both AST and ALT significantly in CCl_4 treated rats indicating maintaince 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[19].

In the present study SMD 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[20]. 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_4 treated rats reveals the severity of hepatopathy. SMD 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.

Histopathological studies also provided supportive evidence for the biochemical analysis. The SMD 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_4.

In conclusion, the results of this study demonstrate that the SMD has a potent hepatoprotective action upon CCl_4 induced hepatic damage in rats.

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