Acta Pharmaceutica Sciencia
48: 213-222 (2006)

Protective Effect of *Momordica dioica* Against Hepatic Damage Caused by Carbon Tetrachloride in Rats

K. Thirupathi, S. Sathesh Kumar, P. Govardhan, B. Ravi Kumar, D. Rama Krishna and G. Krishna Mohan*

University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506 009, India

Abstract

The methanolic extract of fruits of *Momordica dioica* (MMD) 300mg/kg and 500mg/kg were studied for hepatoprotective action in male Wistar rats by inducing liver damage using carbon tetrachloride (CCl_4). The protective effect of MMD was compared with the standard drug silymarin. Various biochemical parameters like aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total serum bilirubin, lipid peroxide, glutathione and total antioxidants levels were evaluated.

The results revealed that the MMD (300mg/kg) significantly reduced the AST (7.24%, $P<0.01$), ALT (7.88%, $P<0.01$), lipid peroxide levels (16.53%, $P<0.05$) and total serum bilirubin (30.23%, $P<0.05$). MMD (500mg/kg) significantly reduced the AST (11.59%, $P<0.001$), ALT (12.59%, $P<0.001$), lipid peroxide levels (24.80%, $P<0.01$) and total serum bilirubin (34.10%, $P<0.01$). The antioxidant parameters, glutathione and total antioxidant levels were increased considerably when compared to the levels of toxic group although they were statistically insignificant. The hepatoprotective activity of MMD (500mg/kg) was comparable with silymarin (100mg/kg). The result suggests that the hepatoprotective action of the MMD may be due to the presence of its phytoconstituents like steroids and triterpenoids.

Keywords: *Momordica dioica;* carbon tetrachloride; serum enzyme levels; antioxidants; hepatoprotective activity.

* Corresponding author e-mail: drgkrishnamohan@yahoo.co.in

Introduction

Liver is the main organ for metabolism and detoxification. Various agents like drugs, alcohol, viruses and many other toxic agents damage the cells of liver. Since ancient periods many herbal medicines were used for treating the liver diseases. In modern medical practice also various herbal-based drugs like silymarin *(Silybum marianum)* and Phyllanthins, polyphenols *(Phyllanthus niruri)* are being successfully used. Hence it becomes essential to explore the plant kingdom for development of new phytotherapeutic agents for liver diseases.

Momordica dioica Roxb. Ex. Willd. (cucurbitaceae) is a dioecious, perennial plant with tuberous roots and it is distributed throughout India (Kirtikar and Basu, 1975). Traditionally it was being used for treating eye diseases, poisoning and fever (Satyavati *et al.*, 1987). Fruits, leaves and tuberous roots are used as a folk remedy for diabetes. The plant was reported to posses antidiabetic (Thirupathi Reddy *et al.*, 2005), analgesic, anticatatonic, post coital antifertility, nematocidal, antiallergic, antimalarial, antifeedant, antibacterial and antifungal activity.

Phytochemical investigations have revealed the presence of traces of alkaloids and ascorbic acid in fruits. Lectins, β-sitosterol, saponin glycosides, triterpenes of ursolic acid, hederagenin, oleanolic acid, α-spiranosterol, stearic acid, gypsogenin, momodicaursenol and some aliphatic constituents were isolated from different parts of this plant (Ghosh *et al.*, 1981).

The aim of the present study is to evaluate the hepatoprotective action of fruits of *Momordica dioica* and to compare its efficacy with silymarin.

Materials and Methods

Plant Material

The fruits of *Momordica dioica* were purchased at the local market of Hanmakonda, Warangal District, Andhra Pradesh in India and it was identified by Dr. Raju S. Vastavaya, Associate Professor, Department of Botany, Kakatiya University,

Warangal. Voucher specimens are being maintained in the herbarium (No.PLB-048) of University College of Pharmaceutical Sciences, Kakatiya University, Warangal. Fresh fruits were washed with tap water, chopped in to pieces and dried in shade. Dried pieces of fruit were ground to coarse powder and stored in an airtight container. The methanolic extract of *Momordica dioica* (MMD) was prepared by the maceration of fruit powder (1000g) with methanol (3L) at room temperature in a round bottom flask for 7 days with intermittent stirring. Then filtered to collect the extract and concentrated under reduced pressure using a rotary flash evaporator (Rotavapor, Buchi, Germany). The extract obtained was preserved in a desiccator to prevent degradation by moisture. A preliminary phytochemical screening was carried out on the methanolic extract by performing tests for alkaloids, glycosides, saponins, flavinoids and steroids. TLC profile of the extract was also studied using chloroform: methanol- (90:10) as solvent system.

Drugs and Chemicals

Silymarin was obtained as a gift sample from Micro Labs (Hosur, Tamilnadu, India). ALT test kit, AST test kit, ALP test kit (Asclepius Immunotek Private Limited), bilirubin test kit (M/s Excel Diagnostics Pvt. Ltd. Hyderadad.), thiobarbituric acid (Hi Media Laboratories Ltd; Mumbai), 1, 1, 3, 3-Tetraethoxy propane (Sigma, st. Louis; USA), trichloro acetic acid (Qualigens Fine chemicals, Mumbai), 5-5^1-Dithiobis-2-nitro benzoic acid (Hi Media Laboratories Ltd, Mumbai), sodium dihydrogen phosphate (S.D. Fine chemicals; Mumbai), glutathione (Hi Media Laboratories Ltd; Mumbai), ascorbic acid (S.D. Fine chemicals; Mumbai), diphenyl picryl hydrazyl (Sigma, St. Louis; USA) are various chemicals and reagents which were used in different stages of the study.

Experimental Animals

Studies were carried out using male Wistar albino rats (180–220 g). They were obtained from the animal house of Mahaveera Enterprises (Reg. No.146/1999/CPCSEA), Ranga Reddy District, India. The animals were grouped and

housed in polyacrylic cages (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. All procedures described were reviewed and approved by the Institutional animal ethical committee.

Hepatoprotective Study

Healthy albino rats were divided into 5 groups each containing 6 animals. Group 1, which served as normal, received 10 ml / kg body weight, p.o of 5% w/v gum acacia in water daily. Group 2 served as toxic and they received 1.5ml/kg body weight of 25% v/v CCl_4 in olive oil on seventh day only. Group 3 received silymarin 100mg/kg body weight of rats per day, Group 4 and 5 received MMD (300 and 500 mg/kg body weight) daily p.o for seven days respectively. On the seventh day, 25% v/v CCl_4 in olive oil was given by oral route at a dose of 1.5ml/kg body weight 30 minutes after the administration of silymarin and extracts. After 36 hours of administration of CCl_4 the rats were anaesthetized with thiopentane sodium (60 mg per kg body weight i.p) and the blood samples were collected from common carotid artery by carefully opening the neck region.

Biochemical Studies

The blood samples were allowed to coagulate at room temperature for at least one hour. Serum was separated by centrifugation at 3000 rpm for 20 minutes and analyzed for AST (Reitman and Frankel, 1957), ALT (Reitman and Frankel, 1957), ALP (Kind *et al.*, 1954), serum bilirubin (Jendrassik and Grof, 1938), glutathione (Beulter, *et.al.*1963), lipid peroxide (Carbonneau *et al.*, 1991) and total antioxidants (Blios, 1958) levels. The results obtained were statistically analyzed by Student's T-test followed by Newman-Keul's multiple comparison tests.

HPLC Conditions for Estimation of Lipid Peroxide Levels:

Mobile phase	:Methanol: Water (70:100) containing 250µl of H_3PO_4 with 80nM of NaOH
Column	:Altec C18 (25 cm length, 4.6 mm diameter, 5µ size)
Wavelength	:540nm
Temperature	:Ambient
Flow rate	:1ml/min.
Injection volume	:20 µl

Results

The preliminary phytochemical screening of the methanolic extracts of MMD indicated the presence of triterpenoids, steroids and glycosides. The TLC studies further confirmed the presence of steroidal and triterpenoidal glycosides by development of violet, blue and green colors on spraying vanillin sulphuric acid reagent.

The effect of MMD on AST, ALT, ALP, serum bilirubin, glutathione, lipid peroxide and total antioxidants levels in CCl_4 induced liver damage in rats are summarized in Table 1. There was a significant increase in AST, ALT, ALP, serum bilirubin and lipid peroxide levels in the toxic group and a sharp decrease in glutathione, total antioxidants levels in rats treated with CCl_4 alone when compared to normal rats. These parameters were positively altered on treatment with MMD300 and MMD500. The therapeutic effects exhibited by these extracts were comparable to standard drug Silymarin (100 mg/kg).

The AST levels were increased to 60.72 U/L in CCl_4 treated rats and are considerably reduced on treatment with MMD300 (56.32 U/L) and MMD500 (53.68 U/L). A significant reduction of $p<0.01$ and $p< 0.001$ were observed in AST levels of MMD 300 and MMD500 groups when compared with toxic group respectively. Dose dependency was exhibited by the extracts. The ALT levels of the rats treated with CCl_4 were found to be increased (82.59U/L) and there was a significant reduction to 76.08 U/L and 72.19 U/L by MMD300 and MMD 500 respectively.

Table 1. Effect of methanolic extract of fruits of *Momordica dioca* on serum enzymes Aspartate amino transferase (AST), Alanine amino transferase (ALT), and Alkaline phospatase (ALP), Total Bilurubin(TBR), Glutathione (GSH), lipid peroxide (MDA) and total antioxidants (TAO) levels in CCl_4 induced liver damage in rats. Values are mean ± S.E.M. number of rats=6. Experimental groups compared with CCl_4 toxic group (* * * $p<0.001$, * * $p<0.01$, * $p<0.05$).

Groups	AST (U/L)	ALT (U/L)	ALP (KAU/ml)	TBR (mg %)	MDA (nM/ml)	GSH (uM/ml)	TAO (uM/ml)
Normal	37.48 (± 3.05)	53.38 (±8.52)	83.63 (±19.54)	0.26 (±0.04)	1.12 (± 0.16)	149.7 (± 42.58)	18.13 (± 3.52)
Toxic	60.72 (± 2.55)	82.59 (±1.90)	128.2 (±2.94)	1.29 (±0.02)	2.54 (± 0.45)	112.8 (± 15.59)	13.82 (±0.77)
Standard	43.48 (± 1.13)	58.46 (±1.80)	89.41 (±20.17)	0.66 (±0.04)	1.41 (± 0.38)	144.2 (± 4.20)	17.93 (±3.50)
MMD300	56.32 (± 1.20)* *	76.08 (± 0.78)**	112.8 (±12.10)	0.90 (±0.10)*	2.12 (± 0.48)*	118.90 (± 5.96)	14.22 (±0.40)
MMD500	53.68 (± 1.40)***	72.19 (±3.44)***	103.6 (±18.99)	0.85 (±0.2)**	1.91 (± 0.41)**	121.8 (± 6.02)	15.03 (± 1.19)

The raise in ALP levels due to induction of hepatotoxicity by CCl_4 were reduced by these extracts, but the values were found to be statistically insignificant ($p>0.05$). The serum total bilirubin levels of hepatotoxicity-induced rats (1.29mg% units) were significantly reduced on treatment by MMD 300 (0.90mg% units) and MMD 500 (0.85mg% units). The GSH levels were decreased to 112.8 µM/ml in toxic group and were considerably recovered, though it was found statistically insignificant.

The crude extract of *Momordica dioica* was also found to posses a mild antioxidant activity, but the values were found to be statistically insignificant ($p>0.05$) when compared with the standard drug.

Discussion

The hepatotoxicity induced by CCl_4 is due to its metabolite $CCl_3^{\bullet}$, a free radical that binds to lipoprotein and leads to peroxidation of lipids of endoplasmic reticulum (Recknagael, 1967). The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin. The lowering of serum enzyme levels is a definite indication of hepatoprotective action of the drug. Protection of hepatic damage caused by carbon tetrachloride administration was observed by recording AST, ALT, ALP and total serum bilirubin levels in treated, toxic and normal groups because serum transaminases, serum alkaline phosphatase and serum bilirubin have been reported to be sensitive indicators of liver injury (Molander *et al.*, 1955). The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane (Zimmerman and Seeff, 1970). This results in decreased levels of AST, ALT and alkaline phosphatase in the hepatic cells and a raised level in serum. The level of lipid peroxidase is a measure of membrane damage and alterations in structure and function of cellular membranes. The level of thiobarbituric acid reactive substance (TBARS) is an indirect measurement of lipid peroxidation (Halliwell *et al.*, 1995). Lipid peroxide levels in tissue were found to be significantly elevated in rats that were treated with CCl_4 alone. This toxic effect is the consequence of CCl_4 activation by cytochrome P-450 to trimethyl radical ($CCl_3^{\bullet}$) which readily reacts with oxygen to form trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$) (Tappel, 1973).

Glutathione is one of the most abundant tripeptide non-enzymatic biological antioxidant present in the liver. Its functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as a peroxidizer in a liver homogenate

that can proceed in a non-enzymatic way. The process is induced by ascorbate in the presence of Fe^{2+}/Fe^{3+}, and it has been reported that Fe^{2+} and ascorbic acid stimulated lipid peroxidation in rat liver microsomes and substrate for glutathione peroxidase and GST (Prakash *et al.*, 2001).

In the present study, sub lethal dose of CCl_4 (1.5ml/kg) of CCl_4 was used to induce hepatotoxicity in rats and the effect of MMD to protect the liver from the toxic effect was investigated by the serum enzyme levels such as AST, ALT, ALP, total serum bilirubin, glutathione, lipid peroxide and total antioxidants.

Momordica dioica was reported to posses steroids (SDS) and triterpinoids (TNS). It was suggested that the plants containing SDS and TNS to posses hepatoprotective and antioxidant activity. The hepatoprotective properties of silymarin have been related to the inhibition of lipid peroxide formations or scavenging of free radicals generated by microsomal ethanol oxidations. To some extent the hepatoprotective activity of MMD can also be related to inhibition of lipid peroxide formations and increase in the total antioxidant levels. Hence this drug can be used in polyherbal formulations to provide a synergistic effect with other hepatoprotective drugs and there by preventing the process of initiation and progress of hepatocellular diseases (Wilkinson, 1962).

The groups treated with MMD 300 and MMD 500 exhibited significant activity on AST, ALT, ALP, TBR and MDA levels in comparison with toxic group. They also showed a mild antioxidant activity by increasing GSH and TAO levels. The increase in dose of the extracts also increased the activity considerably.

The presence of steroids and some triterpenoids in the MMD may be responsible of the protective effect of this plant on liver cells.

References

Ali, M. and Srivatava, V. (1998). Characterisation of phytoconstituents of the fruits of *Momodica dioica*. *Ind. J. Pharm.Sci.* 60:287-289.

Beulter, D.V., Durm, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione. *J. Lab. Chem. Med.* 61(5):882-888.

Blios, MS. (1958). Antioxidant determinations by the use of a stable free radical. *Nature* 26:1199.

Carbonneau, M.A., Peuchant, E., Sess, D., Canioni, P. and Clerc, M. (1991). Free and bound MDA adduct by HPLC in serum and plasma. *Clinc. Chem.* 37 (8): 1423-1429.

Ghosh, P.N., Dasgupta, B. B. and Sircar, P.K. (1981). Purifiction of lectin from a tropical plant *Momordica dioica* Roxb. *Ind. J. Exp. Biol.* 19:253-255.

Halliwell, B., Aeschbach, R., Loligger, J. and Aruoma, O.I. (1995). The characteristic of antioxidants. *Fed. Chem. Toxicol.* 33:601-617.

Jendrassik, L. and Grof, P. (1938). Estimation of total serum bilirubin level by spectrophotometrically in serum and plasma. *Biochem. Z.* 297:81-89.

Kind, P.R.M. and King, E.J. (1954). Estimation of serum alkaline phosphatase activity by colorimetric method. *J. Clin. Path.* 7:322-327.

Kirtikar, K.R. and Basu, B.D. (1975). Indian Medicinal Plants, volume II, India Bishen mahendra pal singh , Dehradun, pp. 842-844.

Luo, L. and Li, Z. (1997). Two new triterpenes of urosolic acids from *Momodica dioica. Yunan Zhiwa Yanji* 19:316-320.

Luo, L., Li, Z., Zhang, Y. and Haung, R. (1998). Triterpenes and steroidal compounds from *Momodica dioica. YaoxeueXuebao* 33:839-842.

Molander, D.W., Wroblewski, F. and La Due, J.S. (1955). Transaminase compared with cholinesterase and alkaline phosphatase an index of hepatocellular integrity. *Clinical Research Proceedings* 3:20-24.

Prakash, J., Gupta, S.K., Kochupillai, Vand Singh, N., Gupta, YK. and Joshi, S. (2001). Chemopreventive activity of *Withania somnifera* in experimentally induced fibrosarcoma tumours in swiss albino mice. *Phytother. Res.* 15:240–244.

Recknagael, R. (1967). Carbon tetrachloride hepatotoxicity. *Pharmacol. Rev.* 19:145-196.

Reitman, S. and Frankel, S. (1957). Colourimetric method for the determination of serum serum transaminases. *Am. J. Clin. Pathol.* 28:56-61.

Satyavati, G.V., Gupta, A.K. and Tandon, N. (1987). Medicinal Plants of India, volume II, ICMR New Delhi, pp. 267.

Tappel, AC. (1973). Lipid peroxidation damage to cell components. *Federal Proceedings* 32:1870-1874.

Thirupathi Reddy, G., Ravi Kumar, B., Krishna Mohan, G. and Mullangi R. (2005). Antihyper glycemic activity of *Momordica dioica* fruits in alloxan induced diabetic rats. *Nigerian J. Natural Products and Medicines* 9:33-34.

Wilkinson, J.H. (1962). An introduction to diagnostic enzymology. In: Arnold. E. (Ed.). Academic Press, London, pp. 1-277.

Zimmerman, H.J. and Seeff, L.B. (1970). Enzymes in hepatic disease. In: Goodly, E.L. (Ed.), Diagnostic Enzymology. Lea and Febiger, Philadelphia, pp. 24-26.

Received: 13.07.2006

Accepted: 27.11.2006