Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 7 (1): 38-45, 2012
ISSN 1816-496X / DOI: 10.3923/jpt.2012.38.45

Efficacy of *Momordica charantia* in Attenuating Hepatic Abnormalities in Cyclophosphamide Intoxicated Rats

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ABSTRACT

Cyclophosphamide (CP) is one among the important therapeutic chemotherapy drug used worldwide. Damage to normal tissues due to toxic metabolites limits the usage of CP efficiently for treating various cancers. In the present study, hepatoprotective effect of *Momordica charantia* Linn. (Cucurbitaceae) against CP induced hepatotoxicity in rats was evaluated. Hepatocellular damage in rats was induced by injecting CP i.p. (total of 200 mg kg^{-1}, b.wt.) for 2 days. *Momordica charantia* fruit aqueous extract (MCE) (300 mg kg^{-1} b.wt.) was administered orally for 12 days for treatment. Protective effect of MCE was evaluated by assessing the liver marker enzymes such as AST, ALT, ALP, LDH and γ-GT in serum, AST and ALT in liver tissues and biochemical parameters such as total protein, urea, creatinine, uric acid, total and direct bilirubin. Liver marker enzymes and clinical chemistry parameters were significantly altered in CP intoxication. These alterations were significantly normalized in animals administered with MCE. Protective effect of MCE could be due to radical-scavenging and antioxidant properties of the MCE. MCE could had been demonstrated these properties due to the presence of phytochemical components that include polyphenols, alkaloids, terpenoids, glycosides and tannins. Present findings supported that the treatment with MCE could be helpful against hepatocellular damage, owing to its hepatoprotective property.

Key words: Hepatoprotective agent, liver marker enzymes, clinical chemistry

INTRODUCTION

Metabolism and detoxification are the main key processes carried out by liver and continuous intoxication of different types of environmental toxic agents could leads to hepatic injury (Zimmerman, 1993). This may leads to the decrease in its efficiency and functions. Cyclophosphamide (CP) is one of the most often used antitumor agents (Cox *et al*., 1976; Hill, 1975). Liver abnormalities were observed in the higher therapeutic administration of CP (Snover *et al*., 1989; Senthilkumar *et al*., 2006a). Acute chemotherapeutic regimens could leads to life-threatening toxicities but are reversible (Suman and Jamil, 2006). An increased interest has been shown around the globe in rediscovering natural sources and food materials that could be helpful as therapeutic agents for the prevention of acute chemotherapeutic injuries.

Natural products and herbal medicines have been used traditionally for various ailments to avoid any side-effects (Abdel-Hamid *et al*., 2011). Phytomedicines become more popular due to its cultural, historical reasons and to meet primary health care requirements (Alakilli, 2009). Natural compounds and indigenous plant based compounds could also have protective effect against CP induced hepatotoxicities (Senthilkumar *et al*., 2006a).

Momordica charantia (Cucurbitaceae) or bitter melon is widely cultivated in Asia, Africa and South America for its valuable medicinal properties (Lotlikar and Rao, 1966). People employ the fruit juice or leaf decoction for diabetes, rheumatism, gout, wounds, infections, hepatitis, laxative and stimulant (Lotlikar and Rao, 1966; Platel and Srinivasan, 1997; Rahman *et al*., 2005; Asiamah *et al*., 2011). Antimicrobial activity was observed in unripe fruit aqueous extract of *M. charantia* and ripening of fruits lead to the loss of its antimicrobial activities (Mahomoodally *et al*., 2010). Chemopreventive effects against precancerous lesions, *in vitro* and *in vivo* antioxidants effect of *M. charantia* were observed in rats (Asiamah *et al*., 2011). Inhibition of skin tumor and mammary tumor development were also observed (Ganguly *et al*., 2000; Nagasawa *et al*., 2002). Biological activities of *M. charantia* could be due to the secondary metabolites content like tannin, flavonoids, terpenoids, glycosides, triterpenes, sterol, resin and phenolic compounds except coumarin and free anthraquinone with minerals such as Mg, Ca, S and Cu (Ullah *et al*., 2011). *M. charantia* acts as an effective immunostimulant during its administration with cow's ghee (Prasad *et al*., 2006). Antibacterial, antineoplastic, antiviral and antimutagenic properties of *Momordica charantia* has been proved in previous studies (Omoregbe *et al*., 1996; Jilka *et al*., 1983; Guevara *et al*., 1990). A literature survey revealed that no research has been carried-out to investigate the protective effect of this plant against CP induced toxicities. Hence, an attempt has been made to screen the hepatoprotective potential of *M. charantia* in CP induced toxicities.

MATERIALS AND METHODS

Drug and plant material: Cyclophosphamide (Ledoxan) was procured from Dabur Pharma limited, New Delhi, India. All other chemicals used were of highest purity and in analytical grade. An authentic sample of *M. charantia* fruits (unripened; usable as a vegetable food) was collected in and around the farms of Thudiyalur, Coimbatore, India. Edible part of the fruit (without seeds) was shade dried and coarsely powdered. Extraction was carried out using warm drinking-water for 30 h to get *M. charantia* aqueous fruit extract (MCE).

Experimental animals: Wistar strain of male albino rats weighing 200±10 g were obtained from animal breeding center, Mannuthi, Kerala were used for the study. Animals were housed in polypropylene cages under standard conditions (25±5°C, humidity 60-70%, 12 h light:dark cycles). Animals were fed with standard pellet diet (AVM cattle and poultry feeds, Coimbatore, Tamil Nadu, India) and drinking water ad libitum. Clearance from Institutional Animal Ethics Committee was obtained prior to the experiment.

Dosage optimization: Different doses of MCE ranging from 100 b.wt. to 500 mg kg^{-1} b.wt. were administered for the study to determine the optimal dose to treat CP intoxication. Dose that has maximum efficiency in a minimum dosage determined by serum marker enzymes for tissue damage was selected as an optimal dose and was found to be 300 mg kg^{-1} b.wt. This dose was selected for the whole of the present study.

Animal treatments: Rats were divided into four groups of 6 animals each:

- **Group I:** Control treated with normal saline
- **Group II:** Toxicity induced with CP (200 mg kg^{-1} b.wt. i.p. on 2 days)
- **Group III:** Rats injected with cyclophosphamide (200 mg kg^{-1} b.wt. i.p. on 2 days) and treated with MCE (300 mg kg^{-1} b.wt. p.o.) for 12 days
- **Group IV:** Administration of MCE alone (300 mg kg^{-1} b.wt. p.o.) for 12 days

At the end of experimental period, the animals were sacrificed by cervical decapitation after overnight fasting. Serum was isolated from blood for various biochemical assays. Liver tissue were immediately washed with ice-cold physiological saline and homogenized in 0.1 M Tris-HCl buffer (pH 7.4) and aliquots were used for the assays.

Biochemical estimations: Liver marker enzymes such as Aspartate Transaminase (AST), Alanine Transaminase (ALT) (Reitman and Frankel, 1957), Alkaline Phosphatase (ALP) (King and Armstrong, 1934), Lactate Dehydrogenase (LDH) (King, 1965), gamma glutamyl transpeptidase (γ-GT) (Thomas, 1998) and the other biochemical parameters such as total protein (Lowry *et al.*, 1951), urea (Natelson *et al.*, 1951), creatinine (Helger *et al.*, 1974) (uric acid (Tietz, 1999), direct and total bilirubin (Tietz, 1976) were analyzed.

Statistical analysis: Data were expressed as Mean±SD. Statistical analyses using SPSS Package (10.1) were done. One way ANOVA followed by post hoc test using Least Significance Difference (LSD) was followed for determining the differences between groups. A $p<0.05$ value was considered statistically significant.

RESULTS

CP was metabolized in the liver by its microsomal enzymes. CP intoxication could leads to abnormal biochemical changes which were reflected in the serum. Table 1 represents the activities of serum marker enzymes AST, ALT, ALP, LDH and γ-GT in control and experimental rats that reflects the tissue damage. In CP challenged rats, compared to control, almost two and three fold increase in the activities of AST and ALT were observed respectively. Also increase in the activities of ALP, LDH and γ-GT were observed in the CP intoxicated rats, compared to control. Administration of MCE resulted in markedly decrease in the activities of marker enzymes. Figure 1 depicts the levels of AST and ALT in the liver of experimental animals. Decrease in the activities of these enzymes in CP toxicity and significant normalization during treatment with MCE was observed. No significant abnormalities were observed in all these enzyme activities in the MCE alone administered group of experimental animals.

Table 1: Effect of MCE extract on serum marker enzymes in the control and experimental rats

Groups	AST	ALT	ALP	LDH	γ-GT
	(IU L^{-1})				
Control	59.02±1.05	32.27±0.92	38.71±0.62	50.36±0.90	57.52±1.47
CP	119.33±1.71*	102.24±1.84*	72.80±0.55*	98.94±1.71*	104.82±2.87*
CP+MCE	61.86±1.33*	43.23±0.93*	52.48±1.07*	70.02±1.58*	63.36±1.85*
MCE	59.05±0.94	31.57±1.05	39.96±1.52	47.84±2.83	56.83±2.18

Results were expressed as Mean±SD (n = 6). Comparisons were made between CP with control, CP+MCE with CP and MCE with control. *Statistically significant at $p<0.05$

Table 2: Effect of MCE extract on biochemical parameters in the control and experimental rats

Groups	Urea	Creatinine	Uric acid	Total bilirubin	Direct bilirubin
	(mg dL^{-1})				
Control	12.17±0.42	0.67±0.01	2.70±0.08	1.40±0.03	0.53±0.06
CP	48.14±1.04*	0.85±0.01*	1.38±0.04*	2.99±0.06*	0.38±0.03*
CP+MCE	13.34±0.24*	0.76±0.02*	2.38±0.07*	1.88±0.06*	0.42±0.02*
MCE	11.93±0.94	0.69±0.03	2.82±0.12	1.25±0.04*	0.50±0.03

Results were expressed as Mean±SD (n = 6). Comparisons were made between CP with control, CP+MCE with CP and MCE with control. *Statistically significant at p<0.05

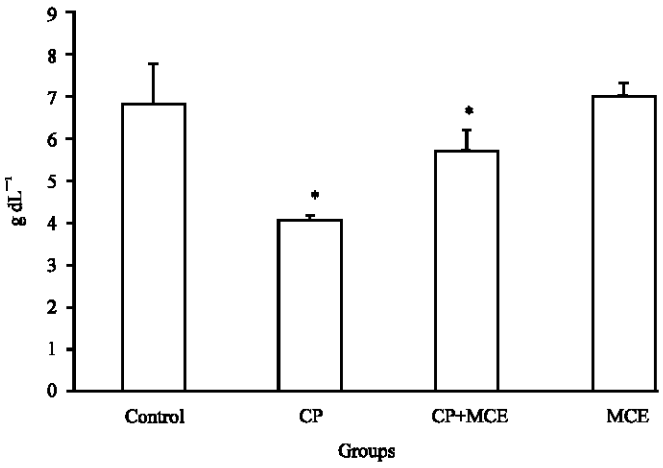

Fig. 1: Effect of MCE extract on serum total protein in the control and experimental rats. Results were expressed as Mean±SD (n = 6). Comparisons were made between CP with control, CP+MCE with CP and MCE with control. *Statistically significant at p<0.05

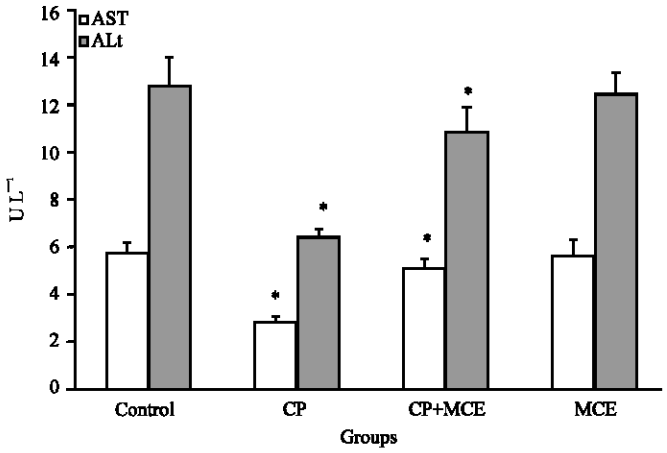

Fig. 2: Effect of MCE extract on AST and ALT in the liver of control and experimental rats. Results were expressed as Mean±SD (n = 6). Comparisons were made between CP with control, CP+MCE with CP and MCE with control. *Statistically significant at p<0.05

Effects of MCE on biochemical parameters were summarized in Fig. 2 and Table 2. Figure 2 showed the decreased level of serum total protein in CP intoxicated rats. Abnormality in the total

protein level has been normalized during MCE treatment. Table 2 summarized the levels of clinical chemistry parameters that were altered during CP intoxication. Abnormally increased levels of total bilirubin, urea and creatinine and decreased levels of uric acid and direct bilirubin due to CP intoxication were significantly normalized during MCE treatment. Also, significantly decreased level of total bilirubin than the control animals were observed in MCE alone administered group that reflects the protective efficacy of MCE towards the normal tissues.

DISCUSSION

Major function of liver is to detoxify xenobiotics and toxins (Mitra *et al*., 1998). The toxic metabolites formed after the administration of CP includes acrolein and phosphoramide mustard, induces liver toxicity in animals (Andrade *et al*., 2007; Arundel and Lewis, 2007; Reuben, 2004; Arora and Goldhaber, 2006; Senthilkumar *et al*., 2006a).

Administration of chemotherapeutic drugs could leads to Single Nucleotide Polymorphisms (SNPs) in chemotherapeutic drug metabolizing enzymes that are responsible for adverse drug reactions (ADR) like alopecia, nausea, vomiting etc. with abnormal liver functions (Khan *et al*., 2007). Cytochrome P450 group of enzymes have extensive functions in liver that includes the detoxification of xenobiotics (Tirona *et al*., 2003). In the present study, elevation of the serum marker enzymes followed by CP intoxication reflected the damage caused to liver. Hepatopathy could leads to the leakage of marker enzymes such as AST, ALT, ALP, LDH and γ-GT into the blood in conformity with the extent of liver damage (Nkosi *et al*., 2005). Also, decreased levels in liver tissues and increased serum levels of both AST and ALT could be due to toxic compounds affecting the integrity of liver cells (Senthilkumar *et al*., 2006a). Prolonged destruction of hepatic cells results in more hepatic release that caused an elevation in serum levels of ALP, LDH (Schmidt, 1978). This could be the reason for the increased serum levels and decreased tissue levels of marker enzymes in the present study. Singh *et al*. (1998) reported that the administration of whole fruit extract of *M. charantia* significantly increased the hepatic levels of Glutathione-S-Transferase (GST) and acid soluble sulfhydryls in lactating dams. Maintenance of enzymic antioxidants could leads to the clearance of toxic oxidants that deteriorate the cellular integrity (Senthilkumar *et al*., 2006b). This could exert protection towards hepatocytes leading to the decline in serum marker enzymes that revealed the ameliorating effect of MCE. Oral administration of methanolic extract of *M. charantia* was safe that was revealed by histopathological observations (Ataman and Idu, 2007). In line with these previous studies, MCE could be considered as safe and efficacious against CP induced hepatotoxicities.

Increased serum urea, creatinine and total bilirubin levels observed in our study were due to the consequences of CP intoxication. Increased blood urea levels concomitant with higher protein catabolic rates have been reported in infections (Latner, 1975). Accumulation of urea could be due to increased catabolism of proteins evidenced by decreased protein level or due to impairment of renal function in CP intoxication. Hematological alterations were observed in CP intoxication (Kailajarvi *et al*., 2000; Senthilkumar *et al*., 2006b). This could be the reason for the increased level of total bilirubin in serum. Observed decreased level of direct bilirubin in our study could be due to the damage to hepatic tissue (Brindha *et al*., 2010). Treatment with MCE normalized the levels of urea, creatinine, direct and total bilirubin near to normal. Protective nature of MCE may be due to polyphenolic and triterpenoids that could have protective role towards the vital organs like liver and kidney (Kang *et al*., 1998; Kumarappan *et al*., 2011).

Preventive antioxidant and chain breaking activities of uric acid has already been documented (Wayner *et al*., 1987). Reduced levels of uric acid in hepatotoxic conditions may be due to the increased utilization of uric acid to combat the released free radicals (Bose *et al*., 2007). This could be the reason for the reported significant fall in uric acid level in CP intoxication and normalization in MCE treatment. All the above findings suggest that MCE could exhibit much pronounced hepatoprotective efficacy during CP intoxication.

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