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In vivo Antioxidant Potential of *Momordica charantia* Against Cyclophosphamide Induced Hepatic Damage in Rats

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ABSTRACT

Cyclophosphamide (CP) is one among the important anti-cancer drug that could cause hepatotoxicity due to its toxic metabolites. In the present study, *in vivo* antioxidant efficacy of *Momordica charantia* against CP induced liver injury in Wistar rats was evaluated. Hepatic damage in rats was induced by injecting CP i.p. (total of 200 mg kg^{-1} b.wt.) for 2 days. Protective effect of aqueous extract of *Momordica charantia* (MCE) was evaluated by assessing enzymic antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase) and lipid peroxidation in liver and non-enzymic antioxidants (glutathione, vitamin C and vitamin E) in serum. Administration of MCE (300 mg kg^{-1} b.wt.) orally for 12 days restored the activities of enzymic antioxidants in liver. Non-enzymic antioxidants were restored to normal level with a concomitant normalized level of lipid peroxidation that could be achieved due to the secondary metabolites, vitamins and minerals content of *Momordica charantia*. Results of the present study evidenced that supplementation of MCE protected hepatic tissues from oxidative damage induced by CP, due to its protective and antioxidant effects.

Key words: Enzymic antioxidants, non-enzymic antioxidants, lipid peroxidation, hepatoprotective agent

INTRODUCTION

Liver is the major site of intense metabolic activities which plays a pivotal role in the removal of substances from the portal circulation that makes it susceptible to damage by offending foreign compounds (Barriault *et al*., 1998). Cyclophosphamide (CP) is the most often applied chemotherapeutic agent in cancer and reported to cause liver injury among antitumor agents (Friedman *et al*., 2003; Senthilkumar *et al*., 2006a). It is catabolised to acrolein and phosphoramide mustard that causes alterations in cell membrane integrity through lipid peroxidation resulting in hepatic damage (Wilmer *et al*., 1990). Medicinal plants and folk medicine usage has been in practice for long time in the treatment of diseases such as jaundice, diabetes, diarrhea, arthritis, skin ulcers, gastrointestinal disturbances etc. (Joseph and Raj, 2011; Sayyad *et al*., 2011; Joseph *et al*., 2012). Plant based natural compounds may provide protection against CP induced toxicities (Senthilkumar *et al*., 2006b; Nithya *et al*., 2012; Sati *et al*., 2010).

Medicinal plants have been reported to contain large amounts of antioxidants that prevent the oxidative stress catalyzed by the free radicals (Velioglu *et al.*, 1998). *Momordica charantia* (bitter melon) is an herbaceous plant belongs to the family Cucurbitaceae which is used as a food as well as medicine (Nithya *et al.*, 2012). Nutritional content of *M. charantia* such as vitamin C and minerals magnesium, calcium, sulphur, copper and other trace elements were documented (Ullah *et al.*, 2011; Ayoola *et al.*, 2010). *In vitro* antioxidant and free radical scavenging activities has been evaluated in the aqueous and ethanolic extracts of wild variety of *M. charantia* (Wu and Ng, 2008). Treatment with bitter melon was found to lower blood glucose levels (Lotlikar and Rajarama Rao, 1966). Extract of this plant exert antibacterial, antineoplastic, antiviral and antimutagenic properties (Guevara *et al.*, 1990). Although, *M. charantia* has been widely used as folk medicine and health food, no scientific investigation had been reported regarding its *in vivo* antioxidant efficacy against CP intoxicated hepatic damage. Therefore, the present investigation has been designed to study the *in vivo* antioxidant potential of aqueous extract of *Momordica charantia* (MCE) in CP induced liver damage.

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. Present study was carried out between August 2007 and April 2008. An authentic sample of *Momordica charantia* fruits (unripened edible part usable as a vegetable food) was collected in and around the farms of Thudiyalur, Coimbatore, India, in the month of August 2007. Edible part of the fruit (without seeds) was shade dried and coarsely powdered. Extraction was carried out using sterile warm drinking-water for 30 h to get 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: MCE dose that have maximum efficiency in a minimum dosage determined by serum marker enzymes for tissue damage was found to be 300 mg kg^{-1} b.wt. (Nithya *et al.*, 2012). This dose was used for the whole of the present study.

Animal treatments: Rats were divided into three 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

No drug control animal group has been maintained due to the non-toxic and edible nature of MCE that has been proved previously (Nithya *et al.*, 2012). At the end of experimental period, the animals were sacrificed by cervical decapitation after overnight fasting. Serum was isolated from blood for 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.

Enzymic and non-enzymic antioxidants assays: Tissue enzymic antioxidants such as superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck *et al*., 1973), Glutathione Reductase (GSH) (Beutler, 1984) and glutathione-s-transferase (GST) (Habig *et al*., 1974) and serum non-enzymic antioxidants that include glutathione (GSH) (Ellman, 1959), Vitamin C (Omaye *et al*., 1979) and Vitamin E (Quaife and Dju, 1949) were analyzed. Lipid peroxidation (Niehaus and Samuelsson, 1968) was determined in the tissue homogenate.

Statistical analysis: Results were expressed as Mean±SD. Significant difference between the groups was analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Least Significant Difference (LSD) test. A $p<0.05$ value was considered statistically significant.

RESULTS

Table 1 represents the effect of MCE on the levels of enzymic antioxidants in the liver of control and experimental groups of rats. Levels of free radical detoxifying enzymes like SOD and CAT were decreased in the liver during CP intoxication. Also, enzymes such as GPx, GR and GST that were involved in the removal of products released by the above enzymes and augmented conjugation with GSH for detoxification were found decreased during CP intoxication. On the contrary, lipid peroxidation in CP intoxicated liver (Fig. 1) revealed a 3-fold significant increase in malondialdehyde (MDA) level, when compared to control group. Supplementation of MCE was able to restore these altered levels of antiperoxidative enzymes and MDA in the liver of experimental

Table 1: Activities of enzymic antioxidants in the liver of experimental groups of animals

Parameters	Control	CP	CP+MCE
Superoxide dismutase (SOD)	11.75±7.42	03.47±0.153*	07.83±0.44*
Catalase (CAT)	106.78±7.42	60.37±4.66*	99.38±4.56*
Glutathione peroxidase (GPx)	36.70±2.67	17.31±1.30*	28.78±2.25*
Glutathione reductase (GR)	1.97±0.064	01.09±0.058*	01.49±0.048*
Glutathione-S-transferase (GST)	66.99±4.30	27.50±1.18*	49.21±1.50*

Results were expressed as Mean±SD, for six rats, SOD: 50% inhibition of nitrite formed/min/mg protein, CAT: μM of H_2O_2 decomposed/min/mg protein, GPx: μg GSH oxidized/min/mg protein, GR: nM NADPH oxidized/min/mg protein and GST: nM CDNB conjugated/min/mg protein, GSH: Reduced glutathione, NADPH : Reduced nicotinamide adenine dinucleotide phosphate, CDNB: 1-Chloro-2,4-dinitrobenzene, *Comparison: CP with control and CP+MCE with CP, Significant at $p<0.05$

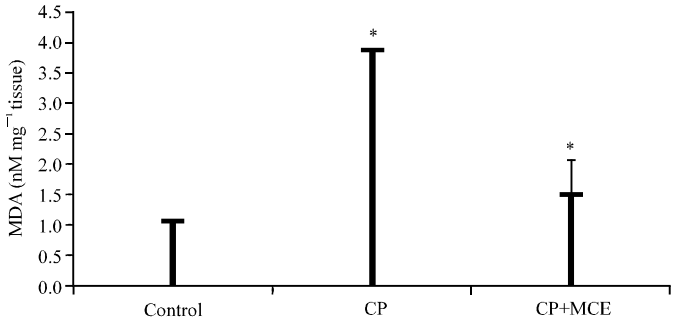

Fig. 1: Levels of MDA in the liver of experimental groups of animals, results were expressed as Mean±SD, for six rats, *Comparison: CP with control and CP+MCE with CP, Significant at $p<0.05$

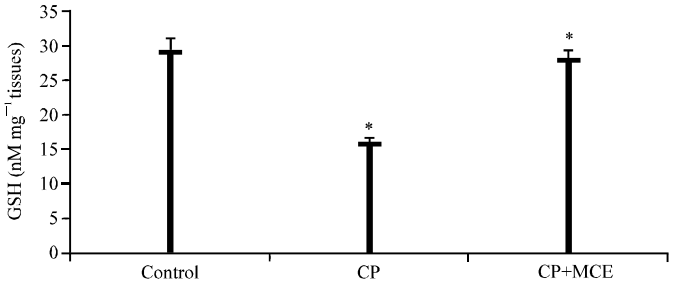

Fig. 2: Levels of GSH in the liver of experimental groups of animals, results were expressed as Mean±SD, for six rats, *Comparison: CP with control and CP+MCE with CP, Significant at $p<0.05$

Table 2: Levels of vitamin C and E in the serum of experimental groups of animals

Parameters (mg dL^{-1})	Control	CP	CP+MCE
Vitamin C	14.60±1.03	8.35±0.930*	11.27±1.050*
Vitamin E	04.62±0.07	2.08±0.087*	04.23±0.012*

Results were expressed as Mean±SD for six rats, *Comparison: CP with control and CP+MCE with CP, Significant at $p<0.05$

animals. Administration of CP significantly decreased the levels of GSH (Fig. 2), Vitamin C and Vitamin E (Table 2) in the serum as compared to control. Treatment with MCE resulted in a significant restoration of the levels of the above non-enzymatic antioxidants.

DISCUSSION

Cyclophosphamide is a commonly used chemotherapeutic drug and well-known mutagen and clastogen (Mohn and Ellenberger, 1976; Edwin *et al.*, 2002). It is an alkylating agent, producing the highly active carbonium ion, which reacts with the extremely electron-rich area of nucleic acids and proteins (Hochstein and Utley, 1968). Oxidative stress induced by CP causes disturbances in the antioxidant state of the cell leads to lipid peroxidation, protein and carbohydrate oxidation and metabolic disorders (Pryor and Godber, 1991; Adesegun *et al.*, 2007). The products of lipid peroxidation such as MDA were toxic to cells causing a devastating effect on the functional status and it is a useful indicator of tissue damage (Raleigh, 1988; Ohkawa *et al.*, 1979).

Increase in LPO levels in liver induced by CP suggests enhanced lipid peroxidation leading to tissue damage and decreased antioxidant defenses in liver (Seven *et al.*, 2004). Treatment with MCE showed significant protection towards these abnormalities. This could be probably due to the hepatoprotective and antioxidant effect of MCE.

SOD scavenges superoxide anion and reduces the toxic and deleterious effects of the free radical. Decreased enzymatic activity of superoxide dismutase is the most sensitive index in hepatocellular damage (Curtis *et al.*, 1972; Jayakumar *et al.*, 2006). Treatment with MCE restored SOD activity suggesting an efficient protective role against Reactive Oxygen Species (ROS). Catalase decomposes H_2O_2 and protects the tissue from highly reactive hydroxyl radicals (Chance *et al.*, 1952). The antioxidant enzymes SOD, CAT and GPx act in coordination to combat the formed ROS (Senthilkumar *et al.*, 2006c) and are directly involved in elimination of free radicals (Sabina and Rasool, 2007). Reduction in the activity may be due to the accumulation of free radicals causing deleterious effects to these enzymes and loss in protective mechanism.

Restoration of enzyme activities near to normal was seen in the treatment of MCE. These findings showed that MCE could be useful to decrease oxidative stress and lower the free radical-mediated tissue damage.

GPx is a selenium dependant enzyme have high potency in scavenging highly reactive free radicals. A specific isozyme, phospholipid hydroperoxide glutathione peroxidase, is the only major antioxidant enzyme that directly reduces phospholipid hydroperoxides within membrane and lipoproteins and act together with alpha-tocopherol (vitamin E) to inhibit lipid peroxidation (Maiorino *et al*., 1991). Decrease in the activity of GPx may be due to the inactivation of enzyme involved and insufficient availability of GSH (Venukumar and Latha, 2002; Senthilkumar *et al*., 2006c). Also, decrease in the activity of glutathione-s-transferase may be due to the depletion in the content of glutathione in liver (Senthilkumar *et al*., 2006a). Glutathione Reductase (GR) is the pivotal enzyme for maintaining and regenerating the reduced levels of glutathione *in vivo* (Senthilkumar *et al*., 2006b). Decrease in the GR activity was observed in CP treatment (Senthilkumar *et al*., 2006c). Restoration of the activities of these enzymes after the administration of MCE supported its protective efficacy.

Glutathione is one of the most abundant non-enzymatic antioxidant present in the cytosol (Valko *et al*., 2007). Its function is concerned with the removal of free radical species (Struznka *et al*., 2005). The depletion of GSH content in serum has to be associated with an enhanced toxicity to chemicals including CP (Reed and Farris, 1984; Senthilkumar *et al*., 2006c). Constituents such as alkaloids, saponin, sterols, steroids, glycosides, pyrimidine nucleoside, triterpenoids, phenolics and flavonoids has been reported to present in *M. charantia* (Raman and Lau, 1996; Chen *et al*., 2005, 2009; Asiamah *et al*., 2011; Ullah *et al*., 2012). Increase in the level of GSH during MCE administration could be due to the presence of above constituents that helped to restore the activities of enzymic antioxidants to prevent the tissue damage.

Vitamin C acts as a direct scavenger of free radicals and reductant in enzymatic reactions (Nagel *et al*., 1997; Saalu *et al*., 2010). Vitamin E is the main lipid soluble antioxidant in membranes preventing hpid peroxidation (Fariss and Zhang, 2003). Vitamin C and Vitamin E acts synergistically in scavenging a wide variety of ROS (Van Acker *et al*., 1996). Decreased levels of vitamin C and vitamin E during CP intoxication could leads to increased free radical damage (Lee, 1999). Significantly decreased level of Vitamin E in CP might be due to the excessive utilization of this antioxidant for the quenching of free radicals produced. The normalized levels of non-enzymic antioxidants could be in-part due to its vitamin C and vitamin E content of MCE (Wu and Ng, 2008; Ullah *et al*., 2011) that helped to regenerate vitamins *in vivo* (Van Acker *et al*., 1996). Glutathione is able to regenerate vitamin C and E to their active form (Valko *et al*., 2007). Increased level of glutathione due to supplementation of MCE could also have contributed to the regeneration of vitamin C and E. These findings supported the antioxidant nature of MCE.

CONCLUSION

Results obtained from the present study indicate that the MCE could be helpful in exerting protection against cyclophosphamide-induced oxidative stress. This study supported that CP intake induces hepatotoxicity evidenced from enhanced lipid peroxidation and depletion of antioxidant reserves, while treatment with MCE reverted the altered antioxidant status, thus supporting its hepatoprotective property.

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