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Carbofuran induced biochemical toxicity in mice: Protective role of *Momordica charantia*

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ABSTRACT

The present study has been designed to evaluate the duration dependent protective effect of aqueous extract of Momordica charantia (MC) and vitamin C against carbofuran (CF) toxicity in liver and kidney tissues of mice. The level of malondialdehyde (MDA), and the activities of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) contents were studied in the liver and kidney tissues. The data showed MDA levels were increased significantly in the CF treated group in 1 and 2 months exposure. But the effect was attenuated by the treatment of MC and vitamin C. The contents of GSH were decreased significantly in the CF treated group in 1 and 2 months exposure. But after treatment GSH contents were increased significantly in MC and vitamin C treated groups. The activities of antioxidant enzymes (SOD and CAT) were also decreased significantly in both the tissues of CF treated group. After treatment with MC and vitamin C activities were significantly increased. These results suggest that the administration of aqueous extract MC and Vitamin C could diminish the adverse effects of carbofuran on lipid peroxidation and activities of antioxidant enzymes in mice.

Keywords: Carbofuran, oxidative stress, mice, *Momordica charantia* and vitamin C.

INTRODUCTION

Wide spread use of pesticide in public health programme and agriculture has caused a severe environmental pollution and potential health hazards. Among all the pesticides used, carbamates are most common. The carbofuran (2,3-dihydro-2, 2-dimethy 1-7-benzofuranyl) is an important organocarbamate pesticide. It is widely used in agriculture and forestry. It is known to produce manifestations of hyperchlolinergic activity in non-target organisms involving central as well as peripheral organs by inhibiting acetylcholinesterase at synapses in the brain and neuromuscular

junctions [1]. The sublethal doses of carbofuran (CF) have also been reported to cause a drastic decrease in total protein content in different tissue viz. liver, heart, brain, muscle, gills and kidney of *Clarias batrachus* (fish) in a dose and duration dependent manner [2]. The acute oral doses of carbaryl have been reported to significantly affect SGPT and SGOT activities in rats [3] and fish [4].

The chronic exposure to CF has been reported to generate reactive oxygen species (ROS) and disturb pro-oxidant/antioxidant balance in rat brain leading to oxidative stress [5, 6]. Mitochondria are the major cellular sources of ROS and key contributors to neurodegenerative disorders. CF treatment has been found to cause significant increase in lipid peroxidation (LPO) [7], induce activities of antioxidant enzymes such as SOD and catalase in rat brain; the impact on catalase being more marked only at high pesticide doses and also causes reduction in protein content of rat tissues tested. It has been clearly demonstrated that rat brain is more severely affected by CF than liver and CF accelerates oxidative stress in rat brain in dose dependent manner [8].

Many Indian plants 10have been reported to possess medicinal properties. *Momordica charantia* (MC), *Asparagus racemosus, Elaeodendron glaucum* Pers., *Magnifers indica, Aegel marmelos* have been reported to possess potent anti-hyperglycemic, hypolipidemic and antioxidant activities [9, 10, 11, 12, 13]; however, their effect on pesticide (carbofuran) induced toxicity has not been studied. Therefore, present investigation was undertaken to assess the effect of an aqueous extract of MC (pulp) and vitamin C on liver and kidney tissues of mice treated with CF induced toxicity in mice for different durations (1 and 2 months).

MATERIALS AND METHODS

Plant material and preparation of extract

MC fruits were purchased from a local vegetable market in Lucknow. Fresh fruits (500 g) were taken and the seeds were removed. The fleshy parts were cut into small pieces and macerated with 500 ml TDW using electrical blander. This suspension was squeezed through a sterile muslin cloth, and the liquid was centrifuged at 5000 rpm for 30 min in the cold. The supernatant was lyophilized at low temperature and reduced pressure by the method of Karunanayaka et al [14] using Christ alpha 1-4, Germany.

Animals and experimental design

Male and female BALB/C mice weighing about 25-30 gram and 5-6 weeks old were purchased from Central Drug Research Institute (CDRI), Lucknow, India, for study and housed at 25±5°C in the animal room in the department. They were provided a standard pelleted diet (Hindustan Lever Ltd, Mumbai, India) and had free access to water. Prior permission for animal use and approval of the protocol were obtained from the CPCSEA, Animal Ethics Committee. After 10 days acclimatization. Animals were divided into four groups viz.

Group I Normal
Group II Carbofuran (0.6 mg/ kg body weight /day)
Group III Carbofuran (0.6 mg /kg body weight/day) + MC extract (13.33 g pulp/kg body weight/day)
Group IV Carbofuran (0.6 mg /kg body weight/day) + vitamin C (150 mg/kg body weight/day)

In each group 12 mice were taken, 6 mice were sacrificed after 1 month treatment and another 6 were sacrificed after 2 months of treatment. Animals were fasted overnight and sacrificed by cervical dislocation causing minimal pain. Liver and kidney tissues were collected in separate ependraff tube and stored at -20°C for study.

Drugs and chemicals

All the drugs and chemicals used in this were purchased from Sigma chemical company Inc., St Louis, Mo, USA. All other chemicals were of analytical grade.

Preparation of tissue homogenate (10% w/v)

Tissues were washed thoroughly with isotonic ice cold solution. The 10% (w/v) homogenates were made using a Potter Elvenhjem homogenizer in ice-cold 50mM phosphate buffer (pH 7.4) containing mammalian protease inhibitor (mention the name and amount used) cocktail. The homogenates were centrifuge at 10,000xg for 30 min at 4^{o}C. The supernatant was used for the assay of antioxidant activities/levels and lipid peroxidation.

Biochemical Profile

Marker of lipid peroxidation viz. malonaldehyde was estimated by method of Ohkawa et al. [15] by using TBA reagent. The results were expressed as nmoles MDA/ 100 gram tissue using 1, 1, 3, 3 tetraethoxypropane (TEP) as reference. The GSH content in tissues was determined by the method of Chandra et al. [16] based on the development of a yellow color when DTNB is added to compounds containing sulfhydryl groups. The activity of superoxide dismutase (SOD) was estimated according to method as described by Misra and Fridovich [17]. One unit of the enzyme activity was expressed as 50% inhibition of auto-oxidation of epinephrine per minute. Activity of catalase (CAT) was assayed by the method of Sinha [18]. One unit of catalase activity was defined as μmoles of H_2O_2 decomposed per min. Protein was estimated by the method of Lowry et al [19] using bovine serum albumin as a standard.

Statistical analysis

The values were expressed as mean ± SEM. The data were subjected to one way Analysis-of-Variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test for comparison between groups and values having $p<0.05$ were considered significant.

RESULTS

Effect on TBARS levels

There was a duration dependent increase in levels of TBARS in liver and kidney tissues of CF treated group. After 1 month exposure the TBARS levels were significantly increased by 29.67% ($p<0.001$) and 49.74% ($p<0.001$) in liver and kidney, respectively, when compared with normal control. After 2 months of exposure, the values were increased up to 72.24% ($p<0.001$) and 88.27% ($p<0.001$) in liver and kidney, respectively. Administration of MC extract with same dose of CF for 1 month resulted in significant decrease in TBARS levels by 18.26% ($p<0.001$) and 20.13% ($p<0.001$) in liver and kidney tissues, respectively. After 2 months of treatment, TBARS levels were decreased by 32.33% ($p<0.001$) and 35.80% ($p<0.001$) in liver and kidney tissues (group III), respectively. Similarly, vitamin C treatment to the CF exposed animals (group IV) resulted in significant decrease in TBARS levels by 12.50% ($p<0.001$), 15.43% ($p<0.001$)

and 35.97% (p<0.001), 43.70% (p<0.001) after 1 month and 2 months treatment, respectively, in liver and kidney tissues (Table 1).

Table 1 : Effect of *Momordica charantia* and Vitmin C on MDA levels in CF treated mice

Groups	MDA levels (nmoles/100g tissue)			
	1 month		2 months	
	Liver	Kidney	Liver	Kidney
I (Normal)	0.802 ± 0.01	0.398 ± 0.006	0.836 ± 0.016	0.430 ± 0.016
II (CF)	1.04 ± 0.02*	0.596 ± 0.013*	1.44 ± 0.02*	0.810 ± 0.009*
III (CF+MC)	0.85 ± 0.02*	0.476 ± 0.006*	0.974 ± 0.009*	0.520 ± 0.016*
IV (CF+VitC)	0.91 ± 0.01*	0.504 ± 0.012*	0.922 ± 0.022*	0.456 ± 0.014*

*All values are expressed as mean ±SEM. (n =12) Group II is compared with Group I and group III and IV are compared with Group II; *p<0.001*

Effect on GSH contents

GSH contents were significantly decreased by 12.02% (p<0.01) and 35.65% (p<0.001) in liver and kidney, respectively, after one month exposure of CF, when compared with normal control. After 2 months of exposure, the values were further decreased by 21.80% (p<0.001) and 39.28% (p<0.001) in liver and kidney, respectively. Administration of MC extract with same dose of CF for 1 month resulted in significant increase in GSH contents by 7.75% (p<0.05) and 44.26% (p<0.001) in liver and kidney tissues, respectively. After 2 months of treatment with MC extract, GSH contents were increased by 22.87% (p<0.001) and 57.84% (p<0.001) in liver and kidney tissues (group III), respectively. Similarly, vitamin C treatment to the CF exposed animals (group IV) resulted in significant increase in GSH contents by 10.61% (p<0.05), 29.61% (p<0.001) and 25.27% (p<0.001), 65.03% (p<0.001) after 1 month and 2 months of treatment, respectively, in liver and kidney tissues (Table 2).

Table 2: Effect of *Momordica charantia* and Vitamin C on GSH levels in CF treated mice

Groups	GSH content (µmoles/mg protein)			
	1 month		2 months	
	Liver	Kidney	Liver	Kidney
I (Normal)	5.57 ± 0.18	4.88 ± 1.23	5.87 ± 0.04	5.04 ± 0.09
II (CF)	4.90 ± 0.06**	3.14 ± 0.05*	4.59 ± 0.06*	3.06 ± 0.05*
III (CF+MC)	5.28 ± 0.08***	4.53 ± 0.08*	5.64 ± 0.1*	4.83 ± 0.05*
IV (CF+VitC)	5.42 ± 0.06***	4.07 ± 0.05*	5.75 ± 0.023*	5.05 ± 0.1*

*All values are expressed as mean ±SEM, (n =12); Group II is compared with Group 1 and group III and IV are compared with Group II; *p<0.001, **p<0.01, ***p<0.05.*

Effect on antioxidants enzyme

As seen in Table 3 and 4 the activities of antioxidant enzymes namely SOD and CAT were significantly decreased (p<0.001) in liver and kidney of 1 month and 2 months CF treated animals (group II) when compared with normal animals (group I). Treatment of CF exposed animals with MC (group III) resulted in significant increase (p<0.001 and p<0.01) in the activities of antioxidant enzymes in liver and kidney tissues of 1 month and 2 months CF exposed animals when compared with CF treated animals (group II). Similarly, vitamin C treatment (group IV) also resulted in significant increase (p<0.001 and p<0.01) in the activities

of antioxidant enzymes in liver and kidney tissues when compared with CF treated animals (group II).

Table 3: Effect of *Momordica charantia* and Vitamin C on catalase activity in CF treated mice

Groups	Catalase activity (U/mg protein)			
	1 month		2 months	
	Liver	Kidney	Liver	Kidney
I (Normal)	14.34 ± 0.18	47.74 ± 0.77	15.19 ± 0.38	49.76 ± 1.05
II (CF)	12.18 ± 0.15*	34.92 ± 0.32*	11.01 ± 0.37*	29.71 ± 0.46*
III (CF+MC)	13.07 ± 0.17**	42.46 ± 0.43*	12.92 ± 0.2*	38.45 ± 0.97*
IV (CF+VitC)	13.52 ± 0.22*	44.76 ± 0.84*	13.48 ± 0.16*	42.92 ± 0.62*

*All values are expressed as mean ±SEM, (n =12); Group II is compared with Group 1 and group III and IV are compared with Group II; *p<0.001, **p<0.01; IU= µmoles H_2O_2 decomposed/ min.*

Table 4 : Effect of *Momordica charantia* and Vitamin C on SOD activity in CF treated mice

Groups	SOD activity (U/mg protein)			
	1 month		2 months	
	Liver	Kidney	Liver	Kidney
I (Normal)	7.9 ± 0.07	14.32 ± 0.52	7.4 ± 0.11	15.38 ± 0.21
II (CF)	6.77 ± 0.11*	10.59 ± 0.19*	5.1 ± 0.05*	10.23 ± 0.31*
III (CF+MC)	7.43 ± 0.08*	13.07 ± 0.17*	6.6 ± 0.17*	13.6 ± 0.22*
IV (CF+VitC)	7.22 ± 0.07**	12.96 ± 0.28*	7.22 ± 0.17*	13.69 ± 0.24*

*All values are expressed as mean ±SEM, (n =12); Group II is compared with Group 1 and group III and IV are compared with Group II; *p<0.001, **p<0.01; IU= 50% inhibition of auto-oxidation of epinephrine/ min.*

DISCUSSION

Carbofuran (CF) is most commonly used in agriculture today. It has been shown to be efficiently absorbed and rapidly redistributed to various organs of non-target organisms as part of their disposal mechanism. Lipid peroxidation may be due to oxidation of molecular oxygen to produce superoxide radicals. This reaction is also the source of H_2O_2, which causes the production of MDA by initiating the peroxidation of unsaturated fatty acids in the membrane. Both H_2O_2 and O_2^- produce highly reactive hydroxyl radical by Haber-Weiss reaction (please check this sentence). The hydroxyl radical can initiate lipid peroxidation which is a free radical chain leading to the perturbations into membrane structure and function [20, 21].The results of present study have shown that CF exposure at subacute dose (0.6 mg/kg body weight) through oral route for 1 month and 2 months caused significant increase ($p<0.001$) in LPO in liver and kidney of CF treated mice. The data suggested that the significantly elevated LPO level in liver and kidney in turn produced ROS, which caused oxidative stress in these organs. Rai et al, [8] also showed that subacute dose of CF exposure may cause increase in LPO in rat liver and brain. After administration of MC extract a significant ($p<0.001$) decrease in LPO levels in liver and kidney tissues were observed at both treatment durations.

The antioxidant enzymes (SOD and catalase) constitute the first line of defense against oxidative stress. Both are the most important defense mechanisms against toxic effects of oxygen metabolism. SOD accelerates the dismutation of H_2O_2, also termed as a primary defense, as it

prevents further generation of free radicals. CAT helps in the removal of H_2O_2 formed during the reaction catalyzed by SOD. Many by-products of oxygen metabolism initiate different outcomes at the subcellular level. The superoxide radicals have been shown to inhibit the activities of glutathioneperoxidase (GPx) and CAT [20]. In addition, singlet oxygen and peroxyl radicals can also inhibit SOD and CAT activities [21]. The data obtained from the present investigation demonstrated the decrease in SOD activity due to the increased production of ROS as evident by the increased LPO levels due to CF treatment. After treatment SOD and CAT activity were significantly increased in liver and kidney in CF treated animals which could be reversed when treated with MC. (Not clear, Match it with the tables 3 and 4).

Present investigation demonstrates that GSH plays an important role in modulating the CF induced oxidative damage in mice. GSH is an important antioxidant system of most aerobic cells [22]. It plays a key role as a cofactor with a variety of enzymes including GPx. GSH depletion has been shown to intensify LPO and predispose cells to oxidant damage [23]. The treatment of CF exposed mice with MC and vitaminC in the present study have significantly restored GSH levels.

In conclusion, the present findings indicate that the CF induces oxidative stress as evidenced by increased levels of lipid peroxidation, decreased GSH contents, and lowered activities of antioxidant enzymes. These results suggest that aqueous extract of *M. charantia* leads to a significant improvement (comparable to non-enzymatic antioxidant, vitamin C) in altered oxidative parameters in mice due to CF treatment.

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Abbreviations:

CAT	*Catalase*
CF	*Carbofuran*
DTNB	*5,5'-Dithio-bis 2-nitrobenzoic acid*
GPx	*Glutathione Peroxidase*
GSH	*Reduced Glutathione*
H_2O_2	*Hydrogen Peroxide*
LPO	*Lipid Peroxidation*
MC	*Momordica charantia*
MDA	*Malondialdehyde*
O_2^-	*Superoxide Radical*
ROS	*Reactive Oxygen Species*
SGOT	*Serum Glutamate Oxaloacetate Transminase*
SGPT	*Serum Glutamate Pyruvate Transminase*
SOD	*Superoxide Dismutase*
TBARS	*Thiobarbituric Acid Reactive Substances*
TEP	*1, 1, 3, 3 Tetraethoxypropane*