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Curcumin mediated attenuation of carbofuran induced toxicity in the heart of Wistar rats

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Abstract: Carbofuran is used to improve the agricultural productivity as well as to protect the house hold and industrial products, but due to accumulation in the biological system, it causes serious side effects in many non-targets mammalian systems. The aim of present study is to evaluate the carbofuran induced oxidative stress in rat heart and its attenuation by using herbal product curcumin. Rats were divided into four groups; one group received 20 % LD_{50} of carbofuran another group of rats received same doses of carbofuran was pretreated with curcumin (100 mg kg^{-1} body weight) and remaining two other groups served as control and curcumin treated animals. The activity of lactate dehydrogenase (LDH) in the heart tissues and serum was evaluated and the activity of enzymatic antioxidants superoxide dismutase (SOD) and catalase (CAT) was estimated in the heart tissues. The level of malondialdehyde (MDA) in heart tissues was also measured. The Total cholesterol (TC) and high density lipoprotein (HDL) was measured in the serum of the entire animals group. The results of present study showed that the activity of LDH in heart tissues were decreased and in serum was elevated. The MDA level was significantly elevated due to exposure of carbofuran. The enzymatic antioxidants, SOD and CAT activities were also inhibited. The ratio of pro-oxidant (P)/antioxidant (A) was also found to be sharply increased in the rat heart tissues of carbofuran exposed animals. The alterations in all the parameter were recovered by the pretreatment of curcumin (100 mg kg^{-1} body weight).

Key words: Curcumin; Carbofuran; Oxidative stress; Antioxidants; Protection; Heart; Total cholesterol.

Introduction

Carbofuran(2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate), is a member of organocarbamate pesticide group, commonly known as Furadan. It is widely used for controlling pests in the agricultural, industrial as well as in the household items due to its broad spectrum action and short half life in the environment (1). Carbofuran reversibly inhibits the AChE activity by cabamylation of hydroxyl group of serine residue which causes accumulation of acetylcholine (ACh) in the synaptic cleft. The relationship between oxidative stress and accumulation of ACh has been experimentally reported by Yang and Dettbarn (1996) (2).

Oxidative stress is defined as the imbalance between free radical production and antioxidant defenses, which results various pathophysiological conditions developed inside the mammalians systems. The toxicity of carbofuran is due to establishment of oxidative stress which has been reported to causes toxicity in various organs of mammalian systems like brain, liver, heart and kidney (3, 4). Various cardiovascular diseases like atherosclerosis, hypertension, heart failure and stroke is related with the oxidative stress (5). Reactive free radicals are highly active, due to presence of unpaired electron which can cause oxidative degradation of protein, lipid and membrane of tissues (6). Cells alleviate the toxic effects of oxidative stress by neutralizing or scavenging the free radicals produced during oxidative stress. The oxidative damage in the cells can be repaired or reduced by the enzymatic antioxidant defense system like superoxide dismutase (SOD), catalase (CAT). The non-enzymatic antioxidants (vitamin E and C, melatonin, flavonoids, etc.) have also been reported to reduce the toxic effect of oxidative stress (7). Lipophilic nature of carbofuran causes accumulation in the fat depots, binding to the biological membrane which causes undesirable toxic effects on to different vital organs such as brain, liver, skeletal muscles and heart as well as lipid peroxidation of biological membrane (8-10).

Turmeric contains a yellow color active herbal polyphenolic ingredient, curcumin. In India, it is commonly used as a spice and as an anti-inflammatory compound in traditional medicine. The therapeutic effects of curcumin are under investigation in well-defined models of disease, like inflammatory hepatic and pancreatic diseases (11). The anticancer property of curcumin has been also reported by suppressing tumor initiation process and metastasis (12, 13). The antioxidant properties of curcumin are due to its phenolics and methoxy groups on the phenyl ring and 1, 3-diketone (14-16).

The antioxidant properties of curcumin have been previously reported in the brain and liver tissues (17). There has been however little attempt to understand the impact of carbofuran on the mammalian heart. Keeping this knowledge gap in mind, it was envisaged in the present study to evaluate the carbofuran induced oxidative stress in heart of the Wistar rat. We have also determined the protective effect of curcumin against carbofuran mediated cardio-toxicity in the experimental animal.

Materials and Methods

Reagents and Chemicals

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate) in the powder form with technical grade 99.6 % purity was a kind gift of Rallis India Limited, Bangalore, India. Edible groundnut oil was purchased from the local market. Pyrogallol, hydrogen peroxide, and bovine serum albumin (BSA) were purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Curcumin was purchased from Sigma-Aldrich Inc. USA. All other chemicals used in the study were of analytical grade.

Animals

Twenty male Wistar rats aged 6 to 7 weeks, weighing 100 to 130 g were purchased from Central Drug Research Institute (CDRI)-Lucknow, India. All the animals were acclimatized for one week at ambient temperature in polypropylene cages in the laboratory. Each cage contains five rats in the laboratory under ambient environmental conditions. They had free access to the food (Dayal Industries Limited-Lucknow, India) and tap water. The experimental procedure was designed according to the guidelines of Institutional Ethical Committee of University of Allahabad.

Treatment of animals with carbofuran and curcumin

Twenty male Wistar rats were divided into four groups; each containing five animals.

(i). Control: Received only 0.5 ml groundnut oil orally up to 15 days at the interval of 24 h.

(ii). 20 % Carbofuran (20 % CF): Received 20 % of LD_{50} (1.6 mg carbofuran kg^{-1} body weight) in 0.5 ml ground nut oil orally up to 15 days at the interval of 24 h.

(iii). Curcumin (Cur): Received 100 mg kg^{-1} body weight curcumin in 0.5ml ground nut oil orally up to 15 days at the interval of 24 h.

(iv). Curcumin + 20 % Carbofuran (Cur + 20 % CF): 100 mg kg^{-1} body weight of curcumin was given orally just before 30 min of carbofuran (20 % LD_{50}), treatment up to 15 days at each interval of 24 h.

At the end of the treatment, all animals were anaesthetized with mild chloroform and sacrificed as per the guidelines of Institutional ethical committee.

Preparation of tissue homogenates for activity assay of antioxidant enzymes and estimation of biomolecules

The heart homogenate (10 % w/v) was prepared in ice cold 0.25 M sucrose solution and centrifuged at 9000 Xg for 30 min at 4-6° C. The supernatants were separated by gentle decantation of centrifuged homogenates of tissues and used for assay of antioxidant enzymes and estimations of the levels of certain biomolecules.

Preparation of serum from rat blood

5 ml blood was collected from each animal by heart puncture in sterile centrifuged tube. The coagulated blood was centrifuged at 1000 g at 4° C and serum was collected as the supernatant.

Estimation of TBARS level

Lipid peroxidation was measured in the cytosolic fraction of heart tissues by following the method of Niehaus and Samuelsson (1968) (18) and the results were expressed as nmol MDA mg^{-1} protein using 1.56×10^5 M^{-1} cm^{-1} extinction coefficient.

Estimation of the activities of antioxidant enzymes

The activity of superoxide dismutase (SOD, E.C. 1.15.1.1) was measured by using the method of Marklund and Marklund (1974) (19). It is spectrophotometric measurement of optical density of colored complex involving pyrogallol auto-oxidation at 412 nm for 3 min at the interval of 30 sec with or without the enzyme protein. One unit of the enzyme activity was expressed as 50 % inhibition of auto-oxidation of pyrogallol min^{-1}.

The catalase (CAT, E.C.1.11.1.6) activity was measured according to the method of Beers and Sizer (1952) (20) by measuring the decrease in the absorbance for H_2O_2 consumption at 240 nm at the interval of 30 sec for 3 min. One unit of CAT activity was defined as µmoles of H_2O_2 decomposed min^{-1} using molar extinction coefficient of H_2O_2 (43.6 M^{-1} cm^{-1}).

Estimation of the activities of Lactate dehydrogenase (LDH) activity

The activity of lactate dehydrogenase (LDH, E.C. 1.1.1.27) was assayed by the method of Horecker and Kornberg (1948) (21) in serum and the cell-free extracts of heart. The reaction was performed in quartz cuvette (1 cm path length). The total reaction mixture (3 ml) contained 1ml 0.2 M Tris-HCl buffer, pH 7.4 , 0.15 ml 0.1 M, KCl, 0.15 ml 50 mM, sodium pyruvate, 0.20 ml 2.4 mM, NADH and suitably diluted enzyme protein. The enzyme activity was monitored as decrease in the absorbance at 340 nm for 3 min. The reaction mixture without enzyme protein served as a control in this assay system.

Estimation of serum lipids

The contents of serum total cholesterol (TC) and high-density lipoprotein (HDL) were measured spectrophotometrically at 560 nm by the method of Zlatkis et al. (1953) (22). Cholesterol was used as standard to determine the value of unknown sample. For HDL measurement, the supernatant of serum treated with phosphotungstic acid and $MgCl_2$ (23) was used and the HDL content was determined according to the method of Zlatkis et al. (1953) (22).

Determination of total protein in the heart

The protein content present in different samples was measured according to the method of Lowry et al. (1951) (24) using BSA as a standard.

Calculation of oxidative stress index

The oxidative stress index has been expressed in terms of the pro-oxidant (P) / antioxidant (A) ratio and was calculated by the following formula:

$$\frac{\text{Levels of MDA}}{\text{Levels of Activity of SOD + Levels of Activity of Catalase}}$$

Statistical analysis

Data are presented as mean ± standard deviation using Graph Pad Prism version 5.01 for Windows,

Graph Pad Software, San Diego California USA. Data were analyzed using one-way analysis of variance (ANOVA). Different groups were compared using Bonferroni's Multiple Comparison Test and considered significant at $p \leq 0.05$.

Results

Effect of carbofuran and curcumin on the activity of LDH in heart and serum of rat

The exposure of rats to repetitive sub-lethal concentration of carbofuran (1.6 mg Kg^{-1} body weight; 20 % of LD_{50} value) up to 15 days at each interval of 24 h showed drastic perturbations in the levels of LDH activity in the rat heart and the serum. The results presented in Figure 1 showed that carbofuran causes significant decrease in the activity of LDH in heart tissues of rat treated with 20 % LD_{50} of carbofuran, the values being 53.43 % as compare to control group. In contrast, the results presented in Figure 2 shows that the activity of LDH got significantly elevated by 86.45 % as compare to control group in the serum of rat under similar experimental conditions.

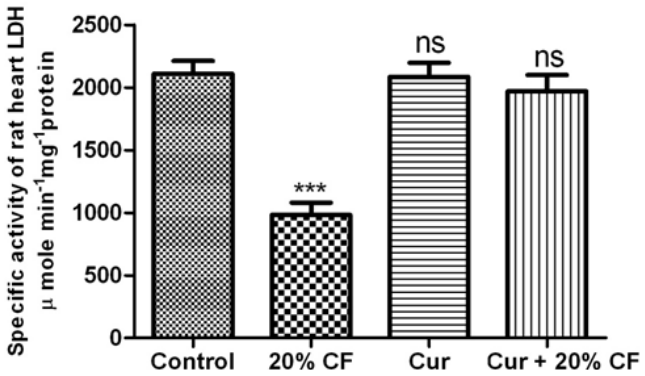

Figure 1. Effect of carbofuran and curcumin on the activity of LDH in rat heart. The procedures for the administration of carbofuran and pretreatment with curcumin as well as assay of LDH activity were the same as mentioned in Materials and Methods. The unit of enzyme activity was expressed as μmole $min^{-1}mg^{-1}$ protein. The data represent mean ±SD of 5 independent experiments. *** indicate the P values significant at <0.001. ns = non significant at P>0.05 as compared to control group.

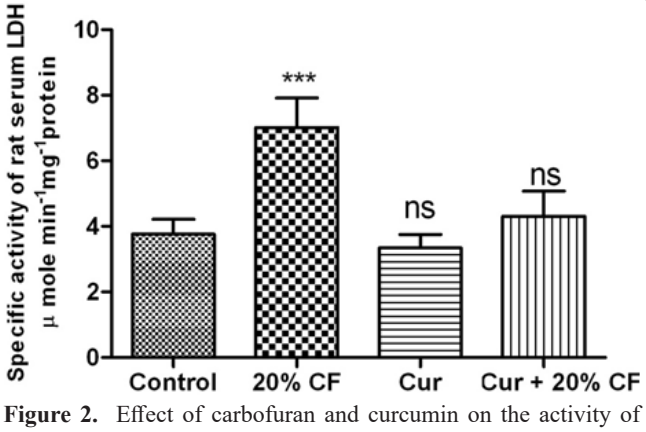

Figure 2. Effect of carbofuran and curcumin on the activity of LDH in the serum of rat. The procedures for the administration of carbofuran and pretreatment with curcumin as well as assay of LDH activity were the same as mentioned in Materials and Methods. The unit of enzyme activity was expressed as μmole $min^{-1}mg^{-1}$ protein. The data represent mean ±SD of 5 independent experiments. *** indicate the P values significant at <0.001. ns=non significant at P>0.05 as compared to control group.

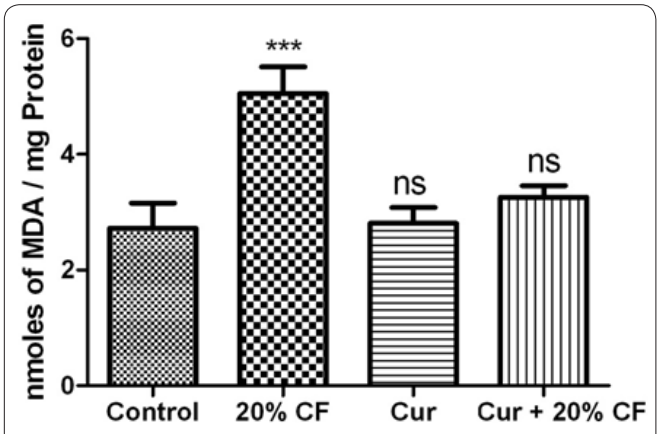

Figure 3. Effect of carbofuran and curcumin on the level of MDA in rat heart. The procedure for the administration of carbofuran and pretreatment with curcumin as well as evaluation of level of MDA in rat heart was the same as mentioned in Materials and Methods. The MDA level was expressed as nmoles of MDA/mg Protein in the rat heart tissues. The data represent mean ±SD of 5 independent experiments. *** indicate the P values significant at <0.001. ns=non significant at P>0.05 as compared to control group.

The animal group pretreated with curcumin (100 mg kg^{-1} body weight) followed by exposure to above concentrations of the carbofuran displayed significant recovery in the level of activity of this enzyme in both heart and serum of rat.

Effect of carbofuran and curcumin on the level of malondialdehyde (MDA) rat heart tissues

The evaluation of the impact of repetitive sub-lethal dose of carbofuran exposure up to 15 days and the ameliorative effect of curcumin was monitored by determining the levels of MDA in the rat heart tissues. The results presented in Figure 3 indicated that carbofuran causes significant increase in the levels of MDA by 85.35 % when compared to control group animals. However, the level of MDA got significantly recovered in the heart tissues of rat pretreated with curcumin followed by exposure to carbofuran.

Effect of carbofuran and curcumin on the activities of antioxidant enzymes (SOD and CAT) in rat heart tissues

The results presented in Figure 4 showed that on the exposure of carbofuran under similar condition as mentioned above, the activity of SOD from experimental rat heart tissues significantly decreased by 56.16 % as compare to control group animals. The activity of catalase in rat heart tissues of same experimental group showed similar type of inhibition as observed in case of SOD. The result presented in Figure 5 indicated significant reduction in the activity of catalase; the values being 46.92 % as compare to control group animals. Upon prior treatment of experimental animals with curcumin, the SOD and catalase activity in rat heart were restored near to the control subjects.

Effect of carbofuran and curcumin on the level of serum lipid (Total cholesterol and HDL) in rat

In order to evaluate the pathophysiological status of heart, the level of total cholesterol and HDL in the

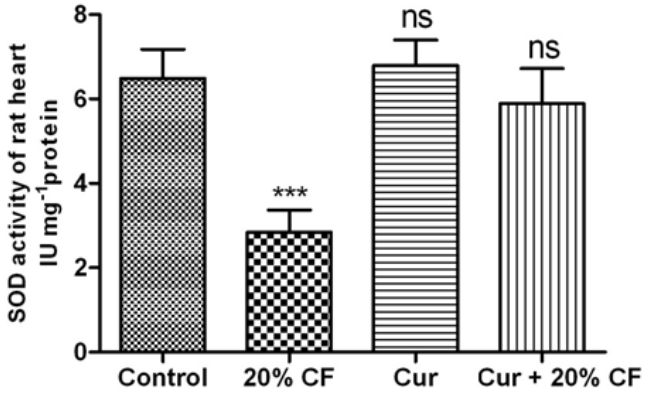

Figure 4. Effect of carbofuran and curcumin on the activity of rat heart SOD. The procedures for the administration of carbofuran and pretreatment with curcumin as well as assay of SOD activity were the same as mentioned in Materials and Methods. The unit of enzyme activity was expressed as IUmg^{-1} protein. The data represent mean ±SD of 5 independent experiments. * and *** indicate the P values significant at <0.001 and <0.05, respectively. ns=non significant at P>0.05 as compared to control group.

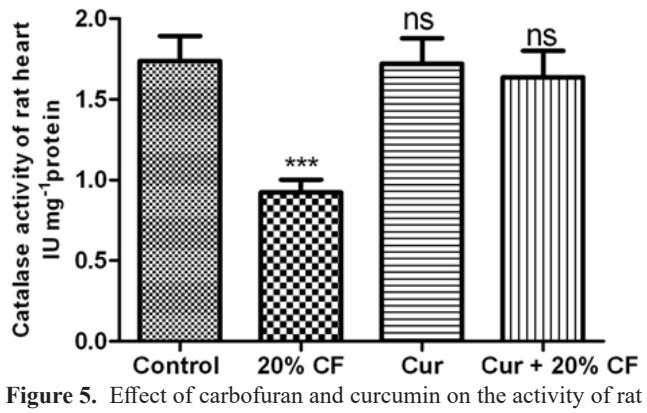

Figure 5. Effect of carbofuran and curcumin on the activity of rat heart catalase. The procedures for the administration of carbofuran and pretreatment with curcumin as well as assay of catalase activity were the same as mentioned in Materials and Methods. The unit of enzyme activity was expressed as IUmg^{-1} protein. The data represent mean ±SD of 5 independent experiments. * and *** indicate the P values significant at <0.001 and <0.05, respectively. ns=non significant at P>0.05 as compared to control group.

serum of rat treated with repetitive sub-lethal dose of carbofuran up to 15 days were measured as shown in materials and methods. The results presented in Table 1 showed that the alterations in the levels of total cholesterol and HDL in the serum due to carbofuran treatment. The pesticide exposure was found to be significantly increase the levels of total cholesterol by 90.7 %, however the level of HDL in the serum of same experimental group got significantly decrease by 74.3 % as compared to control group animal. When the experimental animals were pre-treated with curcumin (100 mg kg^{-1} body weight) followed by exposure to carbofuran, the levels of total cholesterol and HDL in the serum of experimental group animals were restored near the control group.

Effect of carbofuran and curcumin on the oxidative stress index in terms of ratio of pro-oxidant (P)/antioxidant (A) in the rat heart

The results presented in the Table 2 showed that the data obtained after calculation of oxidative stress index in terms of ratio of pro-oxidant (P)/antioxidant (A) in the rat heart was higher in the treated animals and on the pretreatment of curcumin in carbofuran treated animals displayed the value of P/A ratio near to that of control group.

Discussion

Carbofuran causes reversible inhibition of AChE, which results into establishment of oxidative stress and causes carbofuran induced toxicity. The results of present study showed that the reduction of LDH in the cardiac tissues and elevation in the serum due to leakage of cardiac membrane. Tonomura et al. (2009) (25) reported LDH as a cardiac biomarker. The similar type of results was also reported by Jaiswal et al., (2013a) (3) in the cardiac tissues at different dose of carbofuran up to 30 days exposure.

During lipid peroxidation, several biomolecules are produced out of them MDA was consider as biomarkers of membrane lipid peroxidation. The elevation in the level of MDA due to carbofuran exposure showed the onset of oxidative stress in animal cardiac tissues. The elevation of MDA level is believed to be via generation of free radicals and production of oxidative stress. The alteration in the level of MDA in cardiac tissues was reported with the same pesticide as well as with different pesticide diazinon and lindane (3, 26-28).

The biological system comprises the antioxidant defense system which reduced the toxic effect of free radicals. The first line of defense is enzymatic antioxidant

Table 1. Effect of carbofuran and curcumin on level of total cholesterol and HDL in serum.

Lipids	Control	20 % CF	Cur	Cur + 20% CF
Total cholesterol[a]	183.9±14.60	349.0±28.75*** (+90.7%)	160.9±15.77 ns	216.5±18.89 ns
HDL[a]	44.29±6.47	11.38±1.58*** (-74.3%)	40.12±2.67 ns	38.54±4.92 ns

[a] mg dl^{-1}, the (-) and (+) signs show decrease and increase, respectively, in the levels of cholesterol and HDL. The values are mean ± SD of five independent experiments. The procedures for estimations of the level of these biomolecules were as mentioned in Materials and Methods. *** indicate the P values significant at <0.001. ns= non significant at p>0.05 as compared to control.

Table 2. Effect of carbofuran and curcumin on the oxidative stress index in the Wistar rat heart expressed as the ratio of pro-oxidant (P)/antioxidants (A).

OSI	Control	20 % CF	Cur	Cur + 20% CF
P/A	0.3309	1.3430	0.3294	0.4321

The oxidative stress index (OSI) was calculated by determining the pro-oxidant (P)/antioxidant (A) ratio as shown in Materials and Methods.

system, which comprises SOD and catalase, SOD catalyses dismutation of superoxide radicals to hydrogen peroxide and the hydrogen peroxide under goes degradation into nontoxic water and oxygen molecule (29). The results of present study showed that the activity of these antioxidant enzymes were significantly inhibited which indicate that carbofuran causes establishment of oxidative stress in the heart of rat. The results of present investigation was supported by the finding of Jaiswal et al 2013a (3), who reported that the activity of SOD and catalase in rat heart was significantly inhibited by the prolonged exposure of carbofuran. The present investigation was also supported by the inhibition of SOD and catalase activity in the kidney of rat treated with carbofuran (30).

The alteration in the level of total cholesterol and HDL in the blood serum of rat treated with carbofuran supports the toxic effect of carbofuran on the cardiac tissues. The similar type of finding has also been reported by Jaiswal et al (2013a) (3) with same pesticide. The data is also corroborated with the finding of Rai et al (2009) (31), who reported that, the pattern of alteration in the level of total cholesterol and HDL in serum, due to exposure of carbofuran and cartap was same as observed in the present study.

The ratio pro-oxidant (P)/antioxidant (A) in the heart tissues of rat treated with carbofuran was elevated which showed that the carbofuran exposure causes generation of free radicals in the rat heart. This showed that heart is highly susceptible to oxidative stress.

The alterations in the redox state by carbofuran and other pesticide have been shown to recover by using antioxidants (8). Curcumin is a potential antioxidant due to presence of phenolics and methoxy groups on the phenyl ring and 1,3-diketone which reduces the toxic effect of free radical by scavenging, hydro-peroxide reduction and stabilization of free radicals into neutral and non toxic chemicals (14-16,32). The impact of carbofuran on the oxidative stress on heart was shown by the elevation in the level of MDA in the heart tissues which was recovered by the pretreatment of curcumin to carbofuran exposed animals. The antioxidant potential against carbofuran induced toxicity was also seen in the recovery of antioxidants enzymes activity SOD and catalase. The ameliorative effect of curcumin has also been seen in the rat treated with sodium arsenite by (33).

The results from the present study demonstrated that carbofuran at sub-lethal doses was able to induce oxidative stress in rat heart. The effect of carbofuran is to be dose dependent. When rats exposed to carbofuran, it caused oxidative damage in the rat heart. The pretreatment of animals with curcumin showed attenuation of carbofuran induced toxicity in heart. The ameliorative effect of curcumin may be due antioxidant potential of this molecule by scavenging the free radicals produced during oxidative stress.

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