ORIGINAL ARTICLE

Studies on the ameliorative effect of curcumin on carbofuran induced perturbations in the activity of lactate dehydrogenase in wistar rats

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Abstract Carbofuran is known to inhibit neurotransmission system of insects. The present study was undertaken to evaluate the possible ameliorative effect of curcumin on carbofuran induced alterations in energy metabolism in brain and liver of rats. The results demonstrate that carbofuran caused a significant inhibition of lactate dehydrogenase (LDH) activity in rat liver but an increase in LDH activity in the brain. Increased LDH activity was also observed in the serum indicating organ damage in treated animals. Carbofuran caused an increase in level of pyruvic acid in rat liver but a decrease in the brain. A decrease in the level of soluble protein was also observed in the tissues studied. Pretreatment of animals with curcumin resulted in significant amelioration of the altered indices. These results indicate that carbofuran at sub lethal concentrations may adversely alter energy metabolism in brain and liver of non-target mammalian systems. Pretreatment of animals with curcumin may exhibit a potential to mitigate the carbofuran induced toxicity.

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1. Introduction

An increasing use of pesticides in the past few years has resulted in their accumulation in biotic and abiotic systems (Milatovic et al., 2006; Zaahkouk et al., 2000). Organocarbamates and organophosphates (OP) are some of the frequently used pesticides (Milatovic et al., 2006). The underlying mechanism of action of OP pesticides involves irreversible inactivation of acetylcholinesterase (AChE) via formation of an ester bond with hydroxyl group of serine residue present at the active site of AChE, which is essential to regulate neurotransmission in insects, animals and humans (Agrawal and Sharma, 2010). Carbamates, on the other hand, cause reversible inhibi-
tion of AChE by carbamylation of serine residue of AChE, exhibiting relatively low toxicity as compared to OP.

Carbofuran (C₁₂H₁₅NO₃; 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), commonly known as furadan, is a carbamate pesticide used in farm practices to increase crop productivity. It is also used as an insecticide, nematicide and acaricide due to its short half life in the environment (Gupta, 1994; Gupta et al., 2000; Jaiswal et al., 2014). Carbofuran being a broad spectrum pesticide is ubiquitously used to protect agricultural and house hold items. However it gets accumulated in the environment and hence adversely affects different organs such as brain, liver, skeletal muscles and heart of even the non-targeted mammalian systems (Gupta, 1994; Jaiswal et al., 2013; Kaur and Sandhir, 2006; Rai et al., 2009). Due to its ubiquitous presence, it has been detected in the maternal plasma, umbilical cord and blood of African–American women and new born babies, respectively (Whyatt et al., 2003).

Though significant research has been done on the cholinergic effects of carbofuran, there is little information on the effect of carbofuran on energy generating pathways. Pesticide treated animals have been shown to display altered level of aerobnic metabolism resulting in perturbed production of ATP through TCA cycle. Pyruvic acid, a metabolite from glycolysis, is utilized in the form of Acetyl-S-CoA in the TCA cycle. Normally, the excess pyruvic acid undergoes a redox chemical reaction catalyzed by lactate dehydrogenase (LDH) using NADH/NAD⁺ towards reversible conversion of pyruvic acid to lactic acid. Perturbations in TCA cycle under influence of pesticide may cause significant changes in the concentrations of pyruvic acid/lactic acid. Pesticides have been reported to inhibit TCA cycle in the mammalian systems (Griffaton et al., 1978; Singh et al., 2009). Pesticides may cause an increase in the concentration of pyruvic acid, which may stimulate LDH activity in the tissues of the treated animals. Gupta et al. (1991) have demonstrated that carbofuran treatment causes perturbations in the total enzyme activity as well isoenzymic profile of LDH. These workers have suggested that membrane damage or membrane leakage may be the possible reason of altered LDH activity in different body tissues of rats treated with carbofuran. However, there is no information available regarding the status of LDH activity in the mammalian systems treated with repeated doses of carbofuran and the possible ameliorative effect of curcumin on carbofuran induced alterations.

Lactate dehydrogenase (LDH EC 1.1.1.27) is a soluble cytosolic enzyme present in most of the living cells. It catalyzes the reversible oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide (NAD⁺) as hydrogen acceptor in the final step in the metabolic chain of anaerobic glycolysis (Nathan et al., 2006a,b; Singh and Sharma, 1998). The anaerobic carbohydrate metabolism occurring via glycolytic pathway is importantly regulated by LDH at its terminal step. LDH is a biomarker widely used in toxicology and in clinical chemistry to diagnose cell, tissue and organ damage (Nathan et al., 2006a,b; Kaplan and Pesce, 1996). Any alteration occurring in the level of LDH activity reflects on to the metabolic changes in the affected tissues (El-Demerdash, 2011; Fetou et al., 2009).

Turmeric is obtained from the rhizome of Curcuma longa which belongs to the family, Zingiberaceae. The yellow color of turmeric is due to active ingredient, curcumin, a polyphenolic herbal ingredient, curcuminoid. Curcumin has been demonstrated to exhibit antioxidant activity against pesticide-induced oxidative DNA damage in human peripheral blood mononuclear cells under in vitro conditions (Ahmed et al., 2011). Curcumin has been also reported to prevent cancer by suppressing tumor initiation process and metastasis. Its application is relatively safe as its toxicity has not been reported even at a dosage of 10 g/day (Aggarwal et al., 2003; Aggarwal and Harikumar, 2009). Turmeric has also been reported to possess anti-inflammatory activity. Curcumin is used as a spice and an anti-inflammatory compound (Dattani et al., 2010; Nanji et al., 2003; Wolkmer et al., 2013). Due to presence of phenolic and methoxy groups on the phenyl ring and 1,3-diketone in curcumin, it acts as a strong antioxidant exhibiting free radical scavenging and metal binding properties (Kakkar and Kaur, 2011; Lee et al., 2010; Yadav et al., 2011). Curcumin has been reported to possess the ability to cross blood brain barrier in mammalian systems (Orlando et al., 2012; Yang et al., 2005) and thereby exert its protective effect on brain. The present study is an attempt to establish the effect of repeated sub-acute doses of carbofuran on LDH activity in two important tissues of the mammalian system viz., brain, liver and serum. The prophylactic effect of curcumin on carbofuran treated rats has also been evaluated in terms of the recovery of LDH activity from inhibition.

2. Materials and methods

2.1. Chemicals

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate) in powered form was a gift from Rallis India Limited, Bangalore India. Groundnut oil was purchased from the local market. Curcumin was purchased from Sigma–Aldrich Inc. USA. Tris, NADH, KCl and sodium pyruvate were purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Bovine serum albumin (BSA), sodium potassium tartrate, copper sulfate and other chemicals were purchased from MERCK-Darmstadt, Germany.

2.2. Animals

Twenty-four male Wistar rats weighing 100–130 g were purchased from Central Drug Research Institute, Lucknow, India. The animals were acclimatized for one week at ambient temperature in polypropylene cages in the laboratory. Each cage contained 6 animals, fed daily with standard pellet purchased from Dayal Industries Ltd. Lucknow, Uttar Pradesh, India. All the experimental procedures were designed according to the Institutional Ethical Committee of the University.

2.3. Treatment of animals with carbofuran and curcumin

After one week of acclimation, the animals were divided into four groups viz., Group 1: control (C) received orally 0.5 ml groundnut oil orally for 6 days at the interval of 24 h, Group 2: Carbofuran treated group (CF) which received 1.6 mg carbofuran kg⁻¹ body weight (20% LD₅₀) in 0.5 ml groundnut oil orally for 6 days at the interval of 24 h, Group 3: Curcumin treated group (Cur) which received 100 mg curcumin kg⁻¹ body weight in 0.5 ml groundnut oil orally 6 days at the
interval of 24 h, Group 4: Curcumin plus carbofuran treated group (Cur + CF) which received 100 mg kg$^{-1}$ body weight curcumin followed by carbofuran (1.6 mg kg$^{-1}$ body weight) after 30 min for 6 days at the interval of 24 h.

2.4. Preparation of tissue homogenates

Rats were sacrificed using mild chloroform anesthesia followed by cervical dislocation 24 h after the last dose of carbofuran. Blood was collected by heart puncture in sterilized centrifuge tubes and allowed to clot to obtain serum which was stored at $-20^\circ$C. Whole brain and liver were dissected out, washed in chilled isotonic saline (0.9% NaCl), blotted to dryness and weighted. The homogenates (10%, W/V) were prepared in 0.25 M sucrose solution and centrifuged at 9000 g for 30 min at 4–6°C. The supernatants were used for determination of LDH activity and protein concentration.

2.5. Determination of LDH activity in the liver, brain and serum

The activity of LDH in cell free extract of brain, liver and serum was measured by the method of Horecker and Kornberg (1948). 3 ml of reaction mixture contained 0.2 M Tris–HCl buffer, pH 7.4, 0.1 M KCl, 50 mM sodium pyruvate, 2.4 mM NADH and suitably diluted enzyme. The enzyme activity was monitored as decrease in the absorbance at 340 nm for 3 min. The enzyme activity was expressed as $\mu$ moles of NADH oxidized min$^{-1}$ mg protein$^{-1}$.

2.6. Determination of pyruvic acid in the liver and brain

The level of pyruvic acid in the brain and liver of rats was determined colorimetrically as described elsewhere (Anthon and Barrett, 2003). The concentration of pyruvic acid was calculated from a standard graph using sodium pyruvate as the standard. Pyruvic acid concentration was expressed as ng mg$^{-1}$ wet tissue.

2.7. Determination of total protein in the liver, brain and serum

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

2.8. 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 $P \leq 0.05$.

3. Results

3.1. Carbofuran induced alterations in pyruvic acid concentration in the brain and liver of rats and amelioration by curcumin

The effect of sub lethal doses of carbofuran on the levels of pyruvic acid in the brain and liver of rats was evaluated to understand the influence of the pesticide on hepatic glycolysis. Table 1 shows the effect of carbofuran and curcumin on the pyruvic acid concentrations in the brain and liver of control and treated rats. The results demonstrate that carbofuran treatment caused a significant increase of 115% ($P < 0.001$) in the level of pyruvic acid in liver when compared to control rats. However a significant decrease of 59% ($P < 0.001$) was observed in the concentration of pyruvic acid in the brain of carbofuran treated rats when compared to control rats. Pretreatment of carbofuran treated rats with curcumin resulted in a significant 44.77% decrease in the level of pyruvic acid in liver when compared to control rats. The results are shown in Table 1 and Figure 1.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Effect of carbofuran and curcumin treatment on LDH activity in the brain of rats. C: control group treated with groundnut oil; CF: carbofuran treated group; Cur: curcumin treated group; Cur + CF: group which received curcumin 30 min prior to carbofuran. The unit of enzyme activity is expressed as $\mu$mole/min/mg protein. The values were expressed as mean ± SD; $n = 6$, where $n$ = number of determinations. ***$P < 0.001$ when compared to control group. *$P < 0.05$ when compared to control group. ns- non significant when compared to control group.

| Tissues   | Control | CF      | Cur    | Cur + CF |
|-----------|---------|---------|--------|---------|
| Brain$^a$ | 114.0 ± 11.34 | 62.99 ± 6.414 *** | 116.3 ± 12.03 ns | 96.90 ± 10.85 ns |
| Liver$^a$ | 170.3 ± 26.65 | 366.6 ± 36.55*** | 158.4 ± 29.85 ns | 208.3 ± 38.62 ns |

$^a$ Pyruvic acid ng mg$^{-1}$ wet weight of tissue.

| Tissues | Control | CF | Cur | Cur + CF |
|---------|---------|----|-----|---------|
| Brain   | 114.0 ± 11.34 | 62.99 ± 6.414 *** | 116.3 ± 12.03 ns | 96.90 ± 10.85 ns |
| Liver   | 170.3 ± 26.65 | 366.6 ± 36.55*** | 158.4 ± 29.85 ns | 208.3 ± 38.62 ns |

ns = non-significant, CF = carbofuran, Cur = curcumin, $n = 6$. All the values were compared with the control.

*** Significant at $P < 0.001$ when compared to control group.
in significant recovery in the levels of pyruvic acid in liver and brain of the treated animals.

3.2. Tissue LDH activity is modulated by carbofuran and curcumin imparts protection

The results presented in Fig. 1 indicate a significant increase \((P < 0.001)\) in LDH activity in rat brain due to carbofuran treatment when compared to that of control rats. Curcumin treatment alone did not have any significant \((P > 0.05)\) effect on the LDH activity in rat brain. However treatment of rats with curcumin followed by carbofuran exposure resulted in significant recovery \((P < 0.05)\) of LDH activity in rat brain.

The effect of carbofuran and curcumin treatments on LDH activity in liver of rats is shown in Fig. 2. Carbofuran treatment of rats caused a significant decrease \((P < 0.05)\) in hepatic LDH activity when compared to that of control rats. Curcumin treatment did not exert any significant \((P > 0.05)\) effect on hepatic LDH activity. However pretreatment of carbofuran exposed rats with curcumin was able to restore LDH activity in liver to near normal levels.

3.3. Carbofuran treatment perturbs serum level of LDH and curcumin affords protection

LDH activity in the serum is normally recognized as a diagnostic marker of tissue damage. Fig. 3 shows the effect of carbofuran and curcumin treatment on serum LDH activity in rats. The results indicate a significant \((P < 0.001)\) elevation of serum LDH activity in carbofuran treated rats when compared to control rats. Curcumin treatment alone caused no significant alteration in serum LDH activity in rats \((P > 0.05)\). However, treatment of rats with curcumin followed by carbofuran treatment resulted in restoration of altered serum LDH activity to almost normal levels.

4. Discussion

4.1. Carbofuran exposure influences the levels of LDH activity in rat tissues and blood

Carbofuran is one of the carbamate groups of pesticides which is known to reversibly inhibit the activity of AChE (Rai et al., 2009; Jaiswal et al., 2013, 2014). Symptoms of overexposure in humans include headache, weakness, abdominal cramping, nausea, blurred vision, convulsion, tremor, and coma. Carbofuran is also toxic to birds, fishes, and bees (Tenenbaum, 2008). The effects of carbofuran have been attributed to accumulation of acetylcholine (Milatovic et al., 2006). The present study demonstrates that carbofuran treatment caused significant changes in LDH activity in the brain, liver and serum of rats. LDH is a marker enzyme of energy metabolism. The significant rise in the LDH activity in rat brain due to carbofuran exposure is noteworthy. Gupta et al. (1991) have demonstrated that a single dose of carbofuran caused significant alterations in the activity of total LDH activities in different tissues (liver, hemidiaphragm, heart and kidney) and the serum of rats. These authors have
suggested that the decrease in enzyme activity could be due to the membrane damage and leakage of LDH caused by carbofuran exposure. They have also shown perturbations in the levels of LDH isozymes in various tissues and the same has been demonstrated to be associated with the differential impact of carbofuran on the corresponding genes responsible for their synthesis under this condition (Gupta et al., 1991). The increased LDH activity in the brain of carbofuran treated rats observed in the present study may be due to the over expression of LDH and its isoenzymes as suggested by Gupta et al. (1991).

### Table 2

| Tissues  | Control       | CF            | Cur            | Cur + CF       |
|---------|---------------|---------------|----------------|---------------|
| Brain²  | 53.34 ± 6.585 | 28.90 ± 6.642 | 53.95 ± 9.648  | 43.23 ± 6.250 |
|         | (−45.81%)     | (−45.81%)     | (−18.95%)      | (−18.95%)     |
| Liver²  | 88.70 ± 7.570 | 55.40 ± 5.152 | 89.39 ± 3.556  | 76.56 ± 7.586 |
|         | (−37.54%)     | (−37.54%)     | (−13.68%)      | (−13.68%)     |
| Serum²  | 84.22 ± 7.100 | 55.56 ± 5.056 | 82.43 ± 8.848  | 72.10 ± 4.284 |
|         | (−34.02%)     | (−34.02%)     | (−14.39%)      | (−14.39%)     |

*ns* = non-significant, CF = carbofuran, Cur = curcumin, n = 6. The values in parenthesis indicate the percent change over control.

² mg g⁻¹ wet weight of tissue.

³ mg ml⁻¹ serum.

*** Significant at *P* < 0.001 when compared to control group.

### 4.2. Proposed mechanism of carbofuran mediated alterations in levels of LDH in rat brain

Though the exact mechanism of action of carbofuran on the LDH activity in rat brain is not known, it appears that carbofuran influences the activity of this enzyme indirectly via acetyl-S-CoA mediated inhibition of pyruvate dehydrogenase complex (Scheme 1). Carbofuran is known to exert a negative impact on the Kreb’s cycle in the rat brain due allosteric inhibition of pyruvate dehydrogenase complex by the presence of pesticides.
excess of unutilized acetyl-S-CoA. Acetyl-S-CoA is an allosteric inhibitor of two key enzymes of pyruvate dehydrogenase complex (PDH) viz., pyruvate dehydrogenase (E1) and dihydriooamide transacytase (E2) (Mathews et al., 2003). The inhibition of E1 and E2 may lead to accumulation of pyruvic acid in the tissues. The increase in pyruvic acid concentration in the liver of carbofuran treated rat may also be due to the inhibition of LDH activity as observed in the present study. The results of this study showed decreased LDH activity in rat liver treated with carbofuran which is in conformity with earlier results (Singh et al., 2009). Gupta et al. (1991) have reported an increase in the activities of various LDH isoenzymes in different parts of rat brain exposed to carbofuran. El-Demerdash (2011) have reported a significant induction of LDH activity by organophosphates and pyrethroids. Inhibition of AChE activity by carbamates has been found to cause unremitting stimulation of nervous tissue and muscle, which, in turn, causes depletion of ATP and phosphocreatine (Milatovic et al., 2006). This in turn can lead to increased glycolysis and a concomitant increase in LDH activity as well as decreased pyruvic acid concentration in the brain of rats treated with carbofuran. It may therefore be proposed that the increase in LDH activity may be a compensatory mechanism to maintain a homeostasis in the concentrations of pyruvic acid and other glycolytic metabolites (Scheme 1). However, the exact mechanism of action of organocarbamates on the energy metabolism of mammalian systems remains unknown.

4.3. Effect of carbofuran on rat liver and serum LDH is different from that observed in the brain

Though carbofuran caused an increase in LDH activity in rat brain, it was found to have an opposite effect on the liver. The results of the present study showed significant decrease in LDH activity in the livers of rats exposed to sub-acute concentration of carbofuran. A sharp decrease in hepatic LDH activity has been demonstrated in chlorpyrifos treated rats (Heikal et al., 2012). Ismail (2013) has reported a significant decrease in hepatic LDH activity in albino male rats treated with malathion. Achudume et al. (2009) have demonstrated the bioaccumulation of pesticides in the muscles, liver and brain of rats. These authors have also demonstrated an inhibition LDH activity in the muscles and liver of pesticide treated animals (Achudume et al., 2009). The decrease in hepatic LDH activity observed in the present investigation may also be due to the damage of hepatocytes causing loss of cellular enzymes including LDH (Bagchi et al., 1995). The decrease in hepatic LDH activity was accompanied by an increase in serum LDH which further confirms this hypothesis. Lactate dehydrogenase (LDH) is a cytoplasmatic enzyme present in all major organs. The extracellular appearance of LDH has been used to detect cell damage (Drent et al., 1996). In the present study, pretreatment of carbofuran exposed rats with curcumin afforded significant protection from the pesticide induced hepatotoxicity and neurotoxicity. Increased serum LDH has also been reported in rats exposed to an organochlorine pesticide, lindane, which was attenuated by omega-3 and Nigella sativa seed oil (Achudume et al., 2009). The protection afforded by curcumin could either due to its antioxidant properties and/or induction of hepatic detoxifying enzymes (Iqbal et al., 2003). The entire cascade of aforesaid biochemical events postulated due to carbofuran treatment has been summarized in the Scheme 1.

4.4. Carbofuran may be interacting at the level of protein biosynthesis or degradation

The results of the present investigation show a significant reduction in the protein content of liver, brain and serum in carbofuran treated rats. A similar decrease in protein content was observed in rats exposed to sodium arsenite (Kaltreider et al., 2001; Nandi et al., 2005). The decrease in the protein content in the studied tissues may be due to binding of carbofuran to glucocorticoid hormone receptor complexes and selectively inhibiting GR-mediated transcription to turn on the genes of protein synthesis (Kaltreider et al., 2001). Alternatively, it could also be increased protein degradation in the tissues. The reduction in plasma protein in animals treated with xenobiotics has been attributed to the changes in metabolism of proteins and free amino acids in the liver. In addition, the low levels of protein detected in the present investigation could be associated to the excessive loss of protein through nephrosis (Yousef et al., 2006).

4.5. Curcumin mediated amelioration of carbofuran induced toxicity in rat

Curcumin is the active ingredient in the dietary spice turmeric (Curcuma longa). Curcumin is a free radical scavenger, hydrogen donor and exhibits both pro- and antioxidant activities. It also binds metals like iron, copper and can therefore functions as an iron chelator. Curcumin is remarkably non-toxic and exhibits limited bioavailability (Hatcher et al., 2008). The adverse effects of carbofuran and other pesticides have been shown to be attenuated by antioxidants (Milatovic et al., 2006). Curcumin (diferuloylmethane) is a phenolic compound which is used for imparting color and flavor to food, possesses antioxidant properties (Sharma, 1976; Araujo and Leon, 2001). In the present study curcumin was observed to alleviate the adverse effects of carbofuran on rat brain. Similar ameliorative effects of curcumin have been described by other workers (Tanwar et al., 2010). Though there are reports indicating the antioxidative and pro-oxidative properties of curcumin at lower and higher doses, respectively, the exact mechanism of interaction of curcumin with LDH is not known (Tanwar et al., 2010).

5. Conclusion

The results of the present study show the toxic effects of carbofuran on the brain, liver and serum of rats. Carbofuran also caused significant alterations in the concentrations of pyruvic acid in the brain and liver of treated rats. This was accompanied by significant alterations in LDH activity and protein content in the brain, liver and serum of carbofuran treated rats. Though the reasons of these changes have been explained above, studies are needed to confirm the hypothesis that increased LDH activity in brain under influence of pesticide is regulated via inhibition of Kreb’s cycle and allosteric regulation of the pyruvate dehydrogenase complex. The amelioration of these toxic effects by curcumin treatment in rats indicates
that this antioxidant compound has potential to mitigate carbofuran induced toxicity in mammalian systems.

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