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Original Article

OXYCAROTENOID LUTEIN REVERSES THE TOXICITY INDUCED BY CARBOFURAN IN WISTAR RATS

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ABSTRACT

Objective: Elucidation of the protective effect of lutein against carbofuran induced toxicity in Wistar rats.

Methods: Male Wistar rats were assigned into 5 groups of five animals. Group I normal received sunflower oil, Group 2 received carbofuran (5 mg/kg b. w.) alone. Group 3-5 received carbofuran plus lutein (50, 100 and 200 mg/kg body weight) respectively. Carbofuran and lutein administration were continued for 14 d. Neurobehavioural markers such as rotarod, grip strength test and pain threshold tests were carried out. After sacrifice, tissues were analysed for marker enzymes, antioxidant enzymes as well as oxidative stress markers.

Results: Low dose of carbofuran was found to produce neurobehavioral problems as seen from the decreased retention time during rotarod test, endurance capacity in grip strength test and increased endurance capacity in pain threshold test. They were found to be significantly reversed by oral lutein administration. Administration of lutein restored the decreased acetylcholinesterase produced by carbofuran. Serum and tissue marker enzymes such as lactate dehydrogenase, creatine kinase and gamma-glutamyltransferase, which were increased by carbofuran were decreased by lutein administration. Lutein administration also reduced oxidative stress parameters which were increased by carbofuran.

Conclusion: The results showed that carbofuran induced toxicity in male Wistar rats was reversed by carotenoid lutein.

Keywords: Carbofuran, Lutein, Acetylcholinesterase, Antioxidant enzymes, Oxidative stress

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INTRODUCTION

Carbofuran (2,3 dihydro 2,2 dimethyl-7-benzofuranol methyl carbamate) (Furadan) (fig. 1) a broad spectrum pesticide commonly is used in agricultural practices [1]. An estimated 5 million pounds of carbofuran are used annually in the United States, 48% of which is used on corn crops [2]. It is one of the most toxic carbamate pesticides and known to exert high toxicity to mammalian systems [3]. It is a systemic insecticide, acaricide, and nematicide which is extensively used for the control of all types of stem borers in rice, sugar-cane, fruits and vegetables. The underlying mechanism of carbofuran induced toxicity is not well understood. Primary mechanism of toxicity of carbofuran is the reversible inhibition of serine group of acetylcholinesterase via carbamylation at the nerve terminals [4], inducing brain hyperactivity, such as convulsions and seizures. The mechanisms involved in the pathogenesis of neuronal damage appear to be linked to free radical-mediated injury [5].

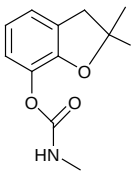

Fig. 1: Structure of carbofuran

Many natural carotenoids have been found to protect the body from the toxicities induced by drugs and carcinogenic xenobiotics [6]. Lutein and zeaxanthin are important carotenoid components of the human diet. The structure of lutein (3,3'-dihydroxy-β, €-carotene) (fig. 2) is characterised by the presence of a hydroxyl group attached to each of the two terminal beta-ionone rings in the molecule. Lutein has been shown to scavenge superoxide radicals, hydroxyl radicals, 2,2-diphenyl–1–picrylhydrazyl radicals and inhibit lipid peroxidation [7]. Lutein has also been shown to induce antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and to increase reducing potential [8]. Research involving cell cultures, animal models, and human studies have shown the potential role of lutein and zeaxanthin in protecting several chronic diseases, particularly age-related macular degeneration (AMD) cataract, cancer at various sites, heart disease, and stroke [9]. Since free radicals are involved in the carbofuran induced toxicity presently we have looked into a reversal of toxicity induced by carbofuran in rats by lutein which is a good free radical scavenger.

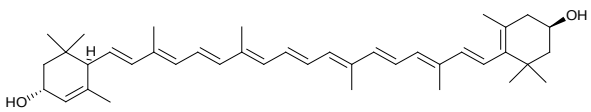

Fig. 2: Structure of lutein

MATERIALS AND METHODS

Chemicals

Lutein isolated from the marigold flower (*Tagetes erecta* L.) by solvent extraction was supplied by Omni Active Health Technologies Pvt. Ltd., Mumbai. Carbofuran was purchased from Sigma-Aldrich (Sample No: 4260085G). Nitrobluetetrazolium (NBT), glutathione (GSH), 5'dithiobis (2-nitrobenzoic acid) (DTNB) and riboflavin were supplied by Sisco Research Laboratries Pvt. Ltd (Mumbai, India). Thiobarbituric acid (TBA) was obtained from Hi-Media Laboratories (Mumbai, India).

Acetyl cholinesterase, gamma-glutamyl transferase, lactate dehydrogenase, creatine kinase activities were measured using biochemical kits supplied by Linear Chemicals (Spain), Agappe

Diagnostics Ltd (Kerala, India), Euro Industrial Chemicals Ltd (India), respectively. All the other reagents and chemicals used were of analytical grade.

Animals

Male Wistar rats (200-300 gm) were obtained from Small Animal Breeding Station (SABS), Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala. They were housed in ventilated cages under controlled conditions of light and humidity and fed with a pelleted diet (Krish Scientific Shopee, Bangalore) and water *ad libitum*. All the animal experiments were done according to the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Sanction No.149/PO/Rc/S/1999/CPCSEA), and were implemented through the Institutional Animal Ethics Committee, Amala Cancer Research Centre.

Reversal of toxicity of carbofuran with lutein

Twenty-five rats were divided into five groups having five animals in each group.

Group 1: Normal (sunflower oil)

Group 2: Carbofuran-5 mg/kg. b. w.

Group 3: Carbofuran-5 mg/kg. b. w.+Lutein-50 mg/kg. b. w.

Group 4: Carbofuran-5 mg/kg. b. w.+Lutein-100 mg/kg. b. w.

Group 5: Carbofuran-5 mg/kg. b. w.+Lutein-200 mg/kg b. w.

Lutein and carbofuran were orally administered once daily in the morning and continued for 14 d. The animals were monitored for clinical and behavioural symptoms such as increased motor activity, tremors, and rolling and arching, clonic convulsions, tonic extension, lacrimation, muscle spasm and the onset of toxicity was noted. Consumption of food was measured on the first day and every third-day intervals throughout the course of the study. Consumption of water for 24 h was also measured similarly for each group of animals. Body weight of rats was also recorded.

Neurobehavioral studies during subacute toxicity

Motor co-ordination studies by rotarod test in rats

Five days before sacrifice, carbofuran induced neurobehavioural changes in experimental rats were analyzed [10] in the presence and absence of lutein. Skeletal muscle relaxation induced by test compounds were evaluated by testing the ability of rats to remain on the rotating rotarod treadmill. The time taken from the start of acceleration period until the rat fell off the drum was measured. The procedure was repeated for 5 consecutive days and average performance time in seconds was taken.

Grip strength test in rats

This test was used to assess the muscular strength in rodents treated with carbofuran with and without lutein. The animals were exposed to a horizontal griddle and made grasp the griddle with forelimbs and hind limbs. The point at which both forelimbs and hind limbs grasp was broken was considered as the time when the animal is fatigued. The time taken until the grasp of both hind and forelimbs were broken was measured which was the endurance capacity of the animal. The procedure was repeated for 5 consecutive days and average endurance capacity in seconds was taken.

Pain threshold test

In this method, the raised tail phenomena in rodents were observed. An artery clip was applied to the root of the tail of rats treated carbofuran with and without lutein to induce pain. The animal quickly responded by biting the tail near the location of the clip. The time between stimulation onset and response was measured by a stopwatch. The procedure was repeated for 5 consecutive days and average pain threshold in seconds was taken for each animal.

Analysis of marker enzymes

At the end of the experiment, all the animals were sacrificed, under light ether anesthesia. Blood was collected directly by heart puncture in non-heparinized vials. The serum was separated by centrifugation at 3000rpm for 10 min. Liver and brain was excised out, washed in normal saline to remove the blood. A portion of liver was fixed in 10% formalin for histopathological analysis. Liver (25% w/v) and brain (25% w/v) homogenate were prepared in Tris-HCl buffer (0.1 M, pH 7.4) and centrifuged at 1000rpm for 30 min at 4 °C. Serum and tissue marker enzymes such as acetylcholine esterase (AChE), creatine kinase (CK), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (γ-GT) were analysed using commercially available kits.

Antioxidant enzymes and glutathione

Superoxide dismutase activity was measured by the NBT reduction method [11]. Glutathione (GSH) levels were assayed based on the reaction with DTNB [12]. The assay of glutathione peroxidase was performed based on the oxidation of GSH in the presence of hydrogen peroxide [13]. Catalase (CAT) activity was estimated by measuring the rate of decomposition of hydrogen peroxide at 240 nm [14].

Determination of oxidative stress

Lipid peroxidation in the liver homogenate was estimated by the thiobarbituric acid method [15]. Tissue hydroperoxides and conjugated dienes were done by the modified method of John and Steven [16]. Total protein in the tissues was estimated by Lowry's method.

Histopathological analysis

A portion of the selected organs (liver and brain) of the control group (carbofuran treated groups) and animals treated highest concentration of lutein (200 mg/kg) along with carbofuran as well as a normal group were fixed in 10% neutral buffered formalin. Embedded organs were cut into slices of 2-4 mm and stained with hematoxylin-eosin and sections were observed under light microscope.

Statistical analysis

The values were expressed as mean±SD. Statistical significance of data was compared between control and experimental groups by one-way ANOVA followed by Dunnet's multiple comparison test (post-hoc) using Instat 3 software (Graph Pad Software, Version 3.05,Inc. La Jolla CA, USA).

RESULTS

General conditions and behaviour

Administration of carbofuran (5 mg/kg body weight) to rats was found to produce salivation; muscle twitch, body tremors and convulsions which were shown within 30 min and persisted for about 1 hr. No mortality was observed at the dose studied.

Body weight

Carbofuran administration for 14 d did not show any abnormal changes in the body weight when compared to the normal rats. Treatment with lutein along with carbofuran did not produce any significant changes in the body weight (Data not shown).

Food and water consumption

Administration of carbofuran did not produce any difference in the food and water consumption when compared with normal animals. Treatment with Lutein did not produce any change in the food and water consumption levels (Data not shown).

Effect of lutein administration on neurobehaviour during carbofuran induced toxicity in male Wistar rats

Rotarod test

Marked impairment in motor function was seen following carbofuran exposure, which is evident by a significant decrease ($P<0.01$) in retention time on rotating rods when compared with normal rats. Lutein along with carbofuran treatment increased the retention time significantly when compared to carbofuran alone treated rats (table 1).

Table 1: Effect of lutein administration on neurobehavioral studies during carbofuran induced toxicity in male Wistar rats

Group	Rotarod test (second)	Grip strength test (second)	Pain threshold Test (second)
Normal (sunflower oil)	13.8±3.16	37.04±8.97	2.46±1.21
Carb 5 mg/kg. b. w.	1.9±0.83*	6.46±1.55**	17.8±3.83**
Carb 5 mg/kg+Lut 50 mg/kg	5.2±1.83*	18.4±1.51**	7.64±1.39**
Carb5 mg/kg+Lut 100 mg/kg	7.6±0.73**	31±4.43**	4.7±1.2**
Carb5 mg/kg+Lut 200 mg/kg	11.2±1.8 2**	33.5±3.83**	2.7±1.16**

Values are mean±SD of 5 animals from each group. P values: *<0.05; **<0.01. Values are mean±SD (*n*=6) ***P*<0.01, Carb: Carbofuran; Lut: Lutein. Significance was calculated against the normal and carbofuran treatment group, as well as carbofuran and lutein, treated groups. Treatment was continued for 14 d.

Grip strength test

Carbofuran treated animals showed a significant decrease (P<0.01) in endurance capacity during grip strength test when compared to normal animals. The endurance capacity was found to be increased in all the test groups after administration of lutein along with carbofuran (table 1).

Pain threshold test

Carbofuran treated animals showed a significant increase (P<0.01) in time taken to respond during pain threshold test when compared to the normal animals. Lutein treatment reduced the response time in rats when compared to the carbofuran alone treatment groups (table 1)

Effect of lutein administration on serum and tissue marker enzymes during carbofuran induced toxicity

The activity of AChE in serum, brain and liver was decreased (P<0.01) in carbofuran treated groups when compared with the normal animals. Treatment of animals with lutein reversed (P<0.01) the toxic effect of carbofuran as seen from the increase in acetylcholinesterase in serum and tissues when compared to the carbofuran alone treated group in a dose-dependent manner (table 2).

Table 2: Effect of lutein administration on acetylcholinesterase (AChE) activity during carbofuran induced toxicity in male wistar rats

Group	Serum	Liver	Brain
Normal (sunflower oil)	3261±239.9	4312±930.5	1101.1±52.3
Carb 5 mg/kg	238±99.0*	683.3±134.8**	697.2±148.2**
Carb 5 mg/kg+Lut 50 mg/kg	996±265.9ns	968±29.4ns	914.1±72.7**
Carb 5 mg/kg+Lut 100 mg/kg	1617±196.4ns	1184.5±77.0**	1232.6±67.4**
Carb5 mg/kg+Lut 200 mg/kg	2153±233.3**	2298.1±313.0**	1207.4±79.1**

Values are mean±SD of 5 animals from each group. P values: *<0.05, Acetylcholinesterase is expressed as (U/mg protein) in tissues and serum it is U/l. Carb: Carbofuran; Lut: Lutein. Significance was calculated against the normal and carbofuran treatment group, as well as carbofuran and lutein, treated groups. Treatment was continued for 14 d.

The levels of CK (table 3), LDH (table 4) and γ-GT (table 5) in serum and tissues were increased in the carbofuran treated groups when compared with the normal animals indicating tissue damage. The levels were significantly (P<0.01) reduced by lutein administration in a dose-dependent manner.

Table 3: Effect of lutein administration on creatine kinase (CK) activity during carbofuran induced toxicity in male Wistar rats

Group	Serum	Liver	Brain
Normal (sunflower oil)	165.8±0.32	124.7±1.16	40.18±3.15
Carb 5 mg/kg	307.2±0.71**	170.7±0.50**	116.8±6.26**
Carb 5 mg/kg+Lut 50 mg/kg	192.0±7.46**	126.4±0.65**	97.86±3.43**
Carb5 mg/kg+Lut 100 mg/kg	236.6±5.27**	111.5±0.92**	77.88±6.41**
Carb5 mg/kg+Lut 200 mg/kg	165.5±0.30**	120.8±1.57**	43.83±4.51**

Values are mean±SD of 5 animals from each group. P values: *<0.05, **<0.01. Creatine kinase is expressed as (U/mg protein) in tissues and serum it is U/l. Carb: Carbofuran; Lut: Lutein. Significance was calculated against the normal and carbofuran treatment group, as well as carbofuran and lutein, treated groups. Treatment was continued for 14 d.

Table 4: Effect of lutein administration on lactate dehydrogenase activity (LDH) during carbofuran induced toxicity in male wistar rats

Group	Serum	Liver	Brain
Normal (sunflower oil)	76.5±4.64	402±8.31	896.9±8.36
Carb 5 mg/kg	148.6±2.21**	1248.5±5.43**	1606±13.26**
Carb 5 mg/kg+Lut 50 mg/kg	133.5±5.66**	661.5±6.65**	767.8±1.19**
Carb 5 mg/kg+Lut 100 mg/kg	96.2±4.38**	621.2±2.05**	843.6±4.88**
Carb 5 mg/kg+Lut 200 mg/kg	63.5±8.99**	516.8±4.23**	888.2±5.78**

Values are mean±SD of 5 animals from each group. P values: *<0.05, **<0.01. LDH is expressed as (U/mg protein) in tissues and serum it is U/l. Carb: Carbofuran; Lut: Lutein. Significance was calculated against the normal and carbofuran treatment group, as well as carbofuran and lutein, treated groups. Treatment was continued for 14 d.

Effect of lutein administration on antioxidant enzymes and GSH level in liver during carbofuran induced toxicity

The levels of antioxidant enzymes SOD, CAT, GPX and GSH in liver tissue were decreased in the carbofuran treated groups when compared to the normal animals and the levels were significantly (P<0.01) enhanced by lutein administration in a dose-dependent manner (table 6).

Inhibiton of carbofuran induced oxidative stress marker by lutein

Markers of oxidative stress; such as lipid peroxidation (table 7), conjugated dieneas as well as tissue hydroperoxides (table 8) were high in the carbofuran treated animals compared with the normal group. These parameters were significantly (P<0.01) reduced to normal levels in lutein treated groups.

Table 5: Effect of lutein administration on gamma-glutamyl transferase activity (γ-GT) during carbofuran induced toxicity in male Wistar rats

Group	Serum	Liver	Brain
Normal (sunflower oil)	70.6±0.25	51.7±0.74	100.1±2.09
Carb 5 mg/kg	107.4±3.09**	106.4±0.36**	112.4±5.75
Carb 5 mg/kg+Lut 50 mg/kg	95.2±0.20**	96.4±0.17**	114.9±1.87
Carb 5 mg/kg+Lut100 mg/kg	84.3±4.60**	70.3±0.23**	114.1±0.38
Carb 5 mg/kg+Lut 200 mg/kg	70.7±0.04**	60.5±0.44**	106.5±4.17

Values are mean±SD of 5 animals from each group. P values: *<0.05, **<0.01. γ-GT is expressed as (U/mg protein) in tissues and serum it is U/l. Carb: Carbofuran; Lut: Lutein. Significance was calculated against the normal and carbofuran treatment group as well as carbofuran and lutein treated groups. Treatment was continued for 14 d.

Table 6: Effect of lutein administration on carbofuran induced changes in antioxidant enzymes and GSH in liver

Group	SOD	CAT	GPX	GSH
Normal (sunflower oil)	1.10±0.1	8.72±0.79	8.46±0.51	23.4±1.54
Carbofuran 5 mg/kg. b. w.	0.25±0.07**	3.75±0.45**	4.45±0.27**	16.05±0.72**
Carb5 mg/kg+Lut 50 mg/kg	0.56±0.05**	4.18±0.26ns	5.8±0.17**	17.6±1.02ns
Carb5 mg/kg. b. w.+Lut 100 mg/kg	0.69±0.03**	6.03±0.30**	7.5±0.23**	21.32±1.51**
Carb5 mg/kg. b. w.+Lut 200 mg/kg	1.00±0.02**	7.45±0.54**	8.44±0.40**	23.28±1.5**

Superoxide dismutase (SOD) unit-U/mg protein, Catalase (CAT) unit-U/mg protein, Glutathione peroxidase (GPX) unit-U/mg protein, Glutathione (GSH) unit n mol/ml. Values are mean±SD from 5 animals in each group. **P<0.01, *P<0.05, significance was calculated against normal and carbofuran group and carbofuran and lutein group, lutein and carbofuran treatment were continued for 14 d.

Table 7: Effect of lutein administration on carbofuran induced lipid peroxidation in male wistar rats

Group	Lipid peroxidation (nmol/mg protein)
Normal (sunflower oil)	1.73±0.09
Carbofuran 5 mg/kg. b. w.	7.01±0.43**
Carbofuran 5 mg/kg. b. w.+Lutein 50 mg/kg. b. w.	4.77±0.05**
Carbofuran 5 mg/kg. b. w.+Lutein 100 mg/kg. b. w.	3.80±0.11**
Carbofuran 5 mg/kg. b. w.+Lutein 200 mg/kg. b. w.	2.55±0.06**

Values are mean±SD of 5 animals from each group. P values: *<0.05; **<0.01. Lipid peroxidation is expressed in nmol of MDA formed per mg protein.

Table 8: Effect of lutein administration on conjugated diene and hydroperoxides in carbofuran treated Wistar rats

Group	Conjugated diene (mM/100g protein)	Hydroperoxides (mM/100g tissue)
Normal (sunflower oil)	1.91±0.10	7.2±0.22
Carbofuran 5 mg/kg. b. w.	2.77±0.04**	13.23±0.08**
Carbofuran 5 mg/kg. b. w.+Lutein 50 mg/kg. b. w.	2.45±0.05**	12.2±0.34**
Carbofuran 5 mg/kg. b. w.+Lutein 100 mg/kg. b. w.	2.16±.002**	9.93±0.46**
Carbofuran 5 mg/kg. b. w.+Lutein 200 mg/kg. b. w.	1.82±0.07**	8.44±0.67**

Values are mean±SD of 5 animals from each group. P values: **<0.01. Conjugated diene is expressed in mmol/100 gm protein, Hydroperoxide is expressed in mmol/100g tissue.

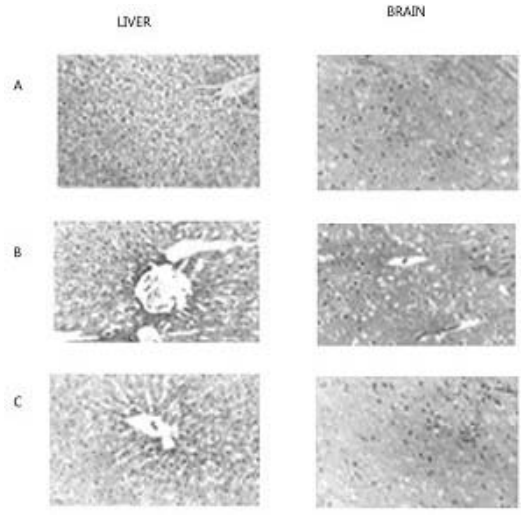

Fig. 3: Histopathology of liver and brain of wistar rats with carbofuran treated rats with and without Lutein, A: Normal, B: Carbofuran alone treated (5 mg/kg body weight), C: Carbofuran (5 mg/kg body weight)+Lutein 200 mg/kg b. w.

Histopathological observations

Histopathology of liver and brain (fig. 3) showed significant damage after carbofuran administration. Liver of normal rats showed well-maintained hepatocytes. In carbofuran treated group, stroma appeared normal, bile duct epithelium denuded, sinuses normal in size but most of the Kupffer cells desorted suggesting haemerragic spots in the parenchyma. Swollen hepatocytes with vacuolated cytoplasm and piknotic nuclei showed dysfunction of liver as a whole. The inflammatory cellular reaction could not be located as a whole in the liver tissue. The cellular changes suggested toxic damage. With lutein treatment, many of the hepatocyte nuclei appeared vesicular nuclei with evident nucleoli suggesting re-generative nuclear changes whereas their cytoplasm remained vacuolated. Brain of normal rats showed normal glial cells. Carbofuran treated brain section showed degeneration of glial cells probably after swelling and degeneration. Many areas showed necrosis. These changes were partially reversed after lutein treatment.

DISCUSSION

Even though carbofuran is banned in several countries; it is still used in many countries to replace other toxic organophosphate pesticides. In fact, the presence of carbofuran residues was reported in the ground, surface water, air, food causing various toxic effects to the non-target organism including humans [17]. The present study evaluates the toxicity of carbofuran administration at non-lethal levels in animal tissue and reversal of its toxicity with carotenoid lutein.

Lutein has been implicated in the prevention of several human health disorders, including macular degeneration, cataracts, cancer, and heart disease. Lutein is one of the members of a family of fat-soluble pigments, is known to be effective chain-breaking antioxidants due to their ability to scavenge reactive oxygen radicals [18]. Numerous studies have reported that lutein showed beneficial effects on antioxidant capacity against reactive oxygen species (ROS) *in vitro* [19].

Pesticides are known to cause free radical-mediated toxicity in organisms via production of ROS. Pesticides induced oxidative stress-mediated toxicity is gradually gaining importance as many classes of pesticides including pyrethroids, organophosphates and organocarbamates have been reported to cause oxidative stress in experimental animals and occupational poisoning [20].

Acetylcholinesterase (AChE), one of the most essential enzymes in the family of serine hydrolases, catalyzes the hydrolysis of the neurotransmitter acetylcholine (Ach), which plays a key role in memory and cognition [21]. Acetylcholine is the neurotransmitter substance released in the synapses between the axon terminals of the postganglionic fibers and the effector organ cells in the case of the parasympathetic system [22]. Cholinesterase is found at the synapses and neuromuscular junctions, where it hydrolyses acetylcholine released at the nerve endings to mediate transmission of impulses. When AChE activity decreases, Ach is not broken and it accumulates within synapses which therefore cannot function in a normal way. In animals and humans, carbofuran toxicity is characterized by rapid inhibition of the activity of AChE through carbamylation of the esteric site of the enzyme leading to cholinergic signs [23]. AChE activity was found to drop rapidly in response to exposure to carbofuran making AChE a good and cost-effective biomarker of chronic carbofuran poisoning indicating its neurotoxicity. The present study demonstrated that there was a restoration in the decreased AChE activity produced by carbofuran in serum and tissues in animals treated with lutein.

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme present in essentially all major organ systems. LDH is found in the form of iso-enzymes. The extracellular appearance of LDH is used to identify cell damage or cell death. It is released into the peripheral blood after cell death by, eg: ischemia, excess heat or cold, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, and from chemical poisonings. A relationship between neoplasia and increased LDH levels has been reported in both human and animal tumors [24]. Increased activity of LDH in serum can be used as an indicator of cellular damage and cytotoxicity of pesticides [25]. LDH is elevated during natural organophosphates or carbamate poisoning [26]. Creatine kinase (CK) is an enzyme expressed by various tissues. CK catalyzes the reversible transfer of the phosphoryl group from phosphocreatine to ADP, to regenerate to ATP. Serum levels of muscle enzyme such as CK is a marker of the functional status of muscle tissue and varies widely in both pathological and physiological conditions. CK plays a key role in the energy metabolism of tissues that have intermittently high and fluctuating energy requirements, such as skeletal and cardiac muscle, and nervous tissue. An increase in this enzymes may represent an index of cellular necrosis and tissue damage following acute and chronic muscle injuries. Serum CK was first used as a diagnostic aid in progressive muscular dystrophy and considered as the main enzyme for the determination of neuromuscular diseases. In our investigation, the increase in CK was dose-dependent.

Data from the present study indicated that the activities of biomarker enzymes like LDH and CK were increased in both serum and in target tissues. Alterations in the activities of biomarker enzymes were reflected in serum as a consequence of their leakage from tissues which are damaged by carbofuran. The leakage of enzymes occurred probably due to substantial depletion of high energy phosphate, ATP, which is essential for retaining intracellular enzymes by maintaining cell membrane integrity and permeability [27]. This study suggests that carbofuran administration not only caused marked depletion of ATP but also reduced energy metabolism inducing muscle hyperactivity.

γ-GT is present in cell membranes of hepatocytes, kidneys, heart, prostate and blood [28]. The primary role of γ-GT is to metabolize extracellular reduced glutathione (GSH), allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis. γ-GT is also involved in the transfer of amino acid across the cell membrane. γ-GT transfers the glutamyl moiety to various acceptor molecule including water, L-amino acids and peptides. Such a process results in the retention of the cysteinyl glycine which was considered to have role in the preservation of intracellular homeostasis during oxidative stress. γ-GT have been commonly associated with liver dysfunction and released into the circulation after cellular damage leading to their elevation in serum [29]. Elevated level of γ-GT has been reported in cardiovascular disease and also in cancer. In our investigation increased activities of these enzymes in serum and tissues could be due to the release of the enzyme from damaged tissues into circulation by the toxic effect of carbofuran. Administration of lutein (50, 100 and 200 mg/kg) for a period of 14 d, prevented the carbofuran induced elevation in the activities of these diagnostic marker enzymes in serum and tissues.

An antioxidant is any substance that when present at low concentrations compared to that of an oxidizable substrate significantly delays or prevents oxidation of that substrate [30]. Antioxidant enzymes comprise the antioxidant defence system of the body against oxidative stress evoked by various xenobiotics. Results from the present study showed that antioxidant enzymes like SOD, GPx, CAT and GSH were reduced in the liver tissue in the carbofuran treated group. This decreased activity may be either due to loss of copper and zinc, which are essential for the activity of the enzyme or due to ROS-induced inactivation of the enzyme [31, 32]. Free radicals react with PUFA of the membrane lipids, initiating lipid peroxidation and produce peroxidation of lipids during oxidative stress, resulting in fragmentation of PUFAs and release of various aldehydes, alkenals, and hydrxyalkenals (eg. MDA) [33]. Oxidative stress markers were increased in the carbofuran treated group which is one of the principle causes of degradation of biomembranes, resulting in leakage of bio membranes, thereby causing an increased enzyme level in the serum. In this study, there was a marked increase in SOD, GPx, CAT and GSH activities in the liver associated with lowering of lipid peroxidation, conjugated dienes and tissue hydroperoxides in the group treated with carbofuran along with lutein in a dose-dependent manner.

As vertical movements in open field are more relevant to know the effects of chemicals on motor activity, the rota-rod test is one of the

tests commonly used in the context of motor function, endurance and balance [34]. During CNS system damage, drug effects on motor coordination or fatigue can be assessed by measuring the time during which the animal remains walking on a rotating drum. In the rota-rod test, the performance of the carbofuran treated animals was impaired compared to the normal rats suggesting its imbalance in motor coordination. Both grip strength and pain threshold test in the carbofuran treated groups rats revealed that there was a significant difference in the performance of rat which may be due to the impairment of the cholinergic system that decreased motor skills and also causes damage to the musculoskeletal and nervous system. Lutein along with carbofuran treatment increased the retention time. The endurance capacity increased in all test groups after administration of lutein along with carbofuran.

CONCLUSION

In conclusion, pesticide carbofuran has been shown to produce severe neurotoxicity in mammals as seen by its inhibition of the activity of acetylcholine esterase. Administration of lutein significantly reduced the neurotoxicity as seen from the increased levels of acetylcholinesterase levels in serum and tissue. Lutein also reduced the tissue toxicity as seen from the decreased levels of creatine kinase, lactate dehydrogenase, γ-glutamyltransferase which were increased by the administration of carbofuran. Carbofuran induced oxidative stress in rats was also reduced by the administration of lutein. Moreover reduced levels of antioxidant enzymes in the tissues and serum produced by carbofuran were significantly enhanced by administration of lutein. The biochemical alterations accompanied by histopathological changes resulted from carbofuran exposures were alleviated following lutein administration. Results are indicative that carbofuran induced toxicity is effectively reduced by lutein administration.

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AUTHORS CONTRIBUTIONS

Dr. Binitha P. P. a Ph. D. student and Ms. Gayathri, a M. Sc. student working at Amala Cancer Research Centre did all the studies. Dr. Ramadasan Kuttan was the main investigator of the project.

CONFLICT OF INTERESTS

The author(s) declare(s) that they have no conflicts of interest to disclose.

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