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Carbamate pesticide carbaryl induces alterations in antioxidant and oxidative stress related responses of Indian buffalo (*Bubalus bubalis*) after sub-chronic exposure

Najeeb Jawad, Rajdeep Kaur, Sreeshitha S Gouri, Vinod K. Dumka, Simrat P. S. Saini*

Department of Pharmacology and Toxicology, College of Veterinary Science,
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana
*spalsaini@yahoo.com

Abstract

The aim of the present work was to study the alterations of the antioxidant defenses and the overall susceptibility to oxidative stress of the *Bubalus bubalis* exposed to the carbamate pesticide Carbaryl at the dose rate of 1mg/kg/day for 105 consecutive days produced no observable signs of toxicity in buffalo calves. However, it produced a significant increase in the extent of lipid peroxidation (42.9%) and in the activity of anti-oxidant enzymes namely glutathione peroxidase (39.2%), glutathione reductase (45.3%), glutathione-S-transferase (29%), superoxide dismutase (65%) and catalase (34.5%).On the other hand, carbaryl administration resulted in significant decrease in the blood glutathione level (26%), an important intra-cellular component of defense against oxidative damage, which is indicative of variations in the overall anti-oxidant profile of buffalo calves induced with sub-chronic carbaryl toxicosis. Overall the current results demonstrated the susceptibility of *B. Bubalis* to oxidative stress induced by the exposure to Carbaryl at an environmentally realistic concentrations, exposing the animal to the risk of oxidative stress damages. The observed responses can be used as sensitive biomarkers for the detection and assessment of effects caused by Carbaryl pesticide exposure.

Keywords: Carbaryl; oxidative stress; buffalo calves; anti-oxidant enzymes

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Introduction

Free radicals and oxidative damage play an important role in cell disintegration and death. Antioxidants alleviate the harmful effects of oxidative damage, which are results of lipid peroxidation. Lipid peroxidation is involved in basic deteriorative mechanisms like membrane damage, enzyme damage and nucleic acid mutagenicity. The formation of lipid peroxides is induced by enzymatic or non-enzymatic peroxidation in the presence of oxygen. In recent years, there has been renewed interest in the role played by lipid peroxidation in drug or pesticide induced oxidative damage. The balance between the formation and removal of lipid peroxides determines the peroxide level in cells and this can be disturbed if cellular defenses are decreased or if there is a significant increase in peroxidative reactions. Once lipid peroxidation is initiated, the reactive intermediate formed induces cell damage.

Carbaryl (1-naphthyl methylcarbamate) is a broad spectrum, general purpose carbamate pesticide, used chiefly as an insecticide. It is used worldwide as a substitute for some organochlorine pesticides (1) and is effective against a range of insects particularly bark beetles, mites, lice, millipedes and other pests and is used for control of insect pests in agricultural and animal husbandry practices (2). It has a very short persistence, which makes it the third most widely used insecticide in the United States for home gardens, commercial agriculture, forestry and range land protection (3). Exposure to

* *Author for correspondence*

carbamates has been linked to free radical generation and thereby oxidative stress and various types of damages to bio-molecules such as DNA, lipids, proteins etc and significant increase in lipid peroxidation and decrease in reduced glutathione (4). Recent studies (5, 6, 7, 8) report that there are stress induced alterations in various anti-oxidant enzyme parameters on exposure to carbaryl in various species of animals.

Materials and Methods

Pesticide & Chemicals

Carbaryl® (Sevin 50% DP) was commercially obtained from Bayer Crop Science Ltd. Mumbai. All chemicals/ reagents used in this study were of analytical grade, obtained from reputed companies.

Animals

The experiment was performed on eight healthy male Murrah buffalo calves of 6-10 months age, weighing between 110-140 kg, at Department of Pharmacology and Toxicology, GADVASU, Ludhiana, Punjab, India. All animals were housed in semi-intensive system and supplemented with the basal diet and water *ad libitum* throughout the experiment. All animals were dewormed prior to study.

Experimental design

After two weeks of acclimatization, animals were randomly divided into two groups of four each. Group 1 served as control whereas the group 2 animals were administered carbaryl orally at the dose rate of 1 mg/kg/ day for 105 days consecutively.

Methods for estimation of antioxidant enzymes

Whole blood samples were collected, from the jugular vein from day 0 to 105 at an interval of 15 days, in clean heparinized centrifuge tubes. Activity of catalase in erythrocyte lysate was determined according to the method described by Aebi (9). The enzyme activity of superoxide dismutase, glutathione-S-transferase, glutathione peroxidase and glutathione reductase in erythrocyte lysate was assayed by the methods described by Marklund and Marklund (10), Habig *et al.* (11), Hafeman *et al.* (12) and Carlberg and Mannervik (13), respectively. Blood glutathione was determined by the method of Beutler *et al.* (14) and lipid peroxidation using the method of Stocks and Dormandy (15).

Statistical Calculations

The differences between two means based on individual observation were determined by student's t-test by using SPSS® 16.0 software. The significance was assessed at $P < 0.05$ and $P < 0.01$ (16).

Results

Carbaryl at the dose rate of 1mg/kg/day for 105 consecutive days resulted in a moderate increase in the extent of lipid peroxidation (Table1) in treatment group from day 15 onwards which became significant from 60th day with an increase of 58.5% on 105th day as compared to the control group. The GSH levels (Table 1) followed a decreasing trend in treatment group and the change became significant from the 45th day onwards with the levels being depleted to an extent of one-fifth of the normal value by 105th day of experiment. However, an increase in the enzymatic activity of glutathione peroxidae (GPx) was observed (Table 1) on 75th, 90th and 105th day of exposure. The increase was at the rate of 23.6%, 39.2% and 26.12% respectively.

Glutathione reductase and glutathione–S-transferase activities (Table 2) increased during the period of exposure to carbaryl, as compared to the control group. This increase of activity in glutathione reductase was significant only on 90th and 105th day of the exposure and was to an extent of 22.7% and 45.3%, respectively as compared to the control group. Highest increase of glutathione–S-transferase activity was observed on 45th day (29%) followed by 105th (22%), 90th (16%) and 75th day (15.8%).

Carbaryl exposure resulted in an increase in the activity of superoxide dismutase (Table 3) in buffalo calves. This increase was 65% on 90th day of carbaryl treatment. Similarly, the enzymatic activity of catalase was also induced in buffalo calves treated with carbaryl (Table 3). This hike in carbaryl exposed animals was significantly different from control animals on 45th day through 60th, 75th, 90th and 105th day to the tune of 34.5%, 9%, 11.5%, 10% and 28.5% respectively.

Table 1. Effect of sub-chronic carbaryl exposure (given at the rate of 1mg/kg for 105 days) on lipid peroxidation, blood glutathione and glutathione peroxidase in buffalo calves

Group	Time (days)							
	0	15	30	45	60	75	90	105
Lipid peroxidation (nmoles MDA/g Hb/ hr)								
Control	7.42 ± 0.81	6.89 ± 0.21	5.89 ± 0.42	6.57 ± 0.41	5.61 ± 0.57	4.45 ± 0.09	7.57 ± 0.53	6.11 ± 0.64
Carbaryl	8.9 ± 0.55	7.27 ± 0.18	7.41 ± 0.58	7.82 ± 0.65	8.02 ± 0.49*	5.01 ± 0.17*	9.71 ± 0.34*	9.69 ± 0.37**
Blood glutathione (µg/ml)								
Control	197.27± 11.11	196.35± 10.89	178.84± 6.10	252.58± 9.58	237.83± 14.32	249.82± 10.25	223.0± 8.31	230.46± 8.71
Carbaryl	192.66± 10.89	188.98± 10.68	140.12± 6.20	214.79± 8.01*	188.05± 7.52*	206.49± 12.50*	202.8± 7.52*	191.74± 2.86*
Erythrocytic glutathione peroxidase (EU/g Hb)								
Control	44.18± 8.02	40.36± 2.44	39.35± 2.93	44.76± 4.78	32.32± 6.68	37.35± 1.5	24.62± 7.81	27.02± 1.21
Carbaryl	52.95± 2.61	57.32± 2.11	45.32± 3.42	48.82± 3.17	37.11± 5.43	46.17± 1.01**	34.27±6.12*	34.07± 1.42**

Values with superscript differ significantly from each other. (*P<0.05 and **P<0.01).

Table 2. Effect of sub-chronic carbaryl exposure (given at the rate of 1mg/kg for 105 days) on glutathione reductase and glutathione-s-transferase in buffalo calves

Group	Time (Days)							
	0	15	30	45	60	75	90	105
Glutathione reductase (oxidation of 1µmole of NADPH/min/gHb)								
Control	12.26± 1.43	16.89± 0.56	17.51± 0.51	14.99± 0.88	14.94± 1.01	12.93± 0.69	13.96± 1.12	10.12± 0.27
Carbaryl	11.81± 0.76	15.66± 0.65	19.23± 2.31	15.89± 1.66	15.91± 1.56	13.73± 0.84	17.14± 0.58*	14.72± 1.25*
Glutathione-S-transferase (µmole of conjugate of GSH and CDNB/min/g Hb)								
Control	357.11±19.51	363.64 ±53.16	370.14 ±22.47	261.98 ±15.41	387.29± 27.02	365.74 ± 10.96	340.18±11.85	252.61±3.27
Carbaryl	367.11 ±15.74	335.55 ±30.31	412.16 ±31.71	337.77±22.2*	405.24 ±41.88	423.04±12.2*	396.05±15.07*	309.11±8.39*

Values with superscript differ significantly from each other. (*P<0.05 and **P<0.01).

Table 3. Effect of sub-chronic carbaryl exposure (given at the rate of 1mg/kg for 105 days) on superoxide dismutase and catalase in buffalo calves

Group	Time (days)							
	0	15	30	45	60	75	90	105
Catalase (µmoles of H2O2 decomposed/min/g Hb)								
Control	3182.57 ± 198.63	3766.19 ± 327.24	2591.43 ± 134.38	3363.54 ± 259.93	3573.95 ± 88.84	2785.65 ± 90.15	2130.46 ± 74.18	2335.59 ±180.47
Carbaryl	3360.41 ± 255.87	3066.87 ± 265.91	2562.52 ± 170.31	4525.49 ± 280.71*	3897.94 ± 87.23*	3105.06 ± 47.58*	2343.96 ± 11.98*	3003.24 ± 27.76*
Superoxide dismutase (amount of enzyme causing 50% inhibition of autoxidation of pyrogallol)								
Control	2.21± 0.11	2.33± 0.54	1.58± 0.11	3.25± 0.15	3.81± 0.21	2.22± 0.21	1.23± 0.16	1.65± 0.13
Carbaryl	2.45± 0.11	1.27± 0.25	1.76± 0.14	3.41± 0.15	4.17± 0.12	2.43± 0.06	2.04± 0.16*	2.33 ± 0.22*

Values with superscript differ significantly from each other. (*P<0.05 and **P<0.01).

Discussion

A significant increase in the extent of lipid peroxidation in carbaryl treated buffalo calves can be substantiated by comparing with the findings of Eraslan *et al.*, (6) and Ozden and Alpertunga (17) in rats. Similarly, this is also in accordance with findings of Meeker *et al.*, (18) in carbamate exposed workers and that of Soderpalm-Berndes and Onfelt (19) in hamster fibroblasts. The spindle disturbing property of carbaryl contributes to the significant increase in the extent of lipid peroxidation (19). So the increase in lipid peroxidation in present study could be due to the oxidative stress inducing ability of carbaryl as a result of its exacerbating action on the lipid bio-membranes.

Glutathione peroxidase catalyzes the reduction of hydroxyl-peroxides by utilizing glutathione (20) and protects against the damaging effect of endogenously

formed hydroxyl-peroxides by reducing them into their corresponding alcohols and also reduces free hydrogen peroxide to water. The measurement of glutathione is considered as a good indicator of antioxidant status or oxidative stress (21). Increased activity of glutathione peroxidase and/or glutathione-S-transferase, superoxide dismutase and higher lipid peroxidation and decreased blood glutathione levels, as seen in this investigation, could be attributed to the utilization of reduced glutathione to remove cytotoxic oxidants spontaneously and non-enzymatically under conditions of carbaryl induced toxicosis. The decrease in the reduced blood glutathione levels are in agreement with observations made by Soderpalm-Berndes and Onfelt (19) who found that carbaryl reduces the glutathione content in Chinese hamster fibroblast. In addition, the findings of Bhunia *et al.* (22), Ghosh *et al.* (23) and Singh *et al.* (18) are in agreement with the findings of the present study.

Elevated activity of glutathione reductase observed in the present study is in accordance with the findings reported by Ribera *et al.* (1) in earthworms, Matos *et al.* in fish (24), Seth *et al.* in rats (25). Glutathione reductase is an enzyme that reduces glutathione disulfide to the sulfhydryl form of reduced glutathione (GSH), which is an important cellular antioxidant (26). It plays an indirect but essential role in the prevention of oxidative damage within the cell by helping to maintain appropriate levels of intracellular glutathione. The regeneration of GSH is catalyzed by glutathione reductase (27) using ß-nicotinamide dinucleotide phosphate (NADPH) as the hydrogen donor (28). Glutathione-S-transferase catalyzes the conjugation of reduced glutathione via a sulfhydryl group to electrophilic centers on a wide variety of substrates (29). This activity detoxifies endogenous compounds such as peroxidized lipids (30), as well as xenobiotics. Glutathione-S-transferase may also bind toxins and function as transport proteins (31). So, the increased activity of glutathione-S-transferase and glutathione reductase could be attributed to the adaptive response of animal to increase metabolism of carbaryl and to withstand the oxidative stress.

The superoxide ion, which has been considered important in lipid peroxidation and the peroxidative hemolysis of red blood cells (32) is formed by the univalent reduction of O_2 during various enzymatic reactions. Carbaryl decreases the sequence of events following the binding of a particulate agent to its receptor and leading to the induction of phospholipase activity and subsequent oxidative burst in the cells (33). In addition, it causes significantly raised met-hemoglobin levels in erythrocytes with 41% of normal SOD activity (34). These data suggest that the increase in superoxide dismutase activity in the present study could be attributed to the increase in the production of superoxide ions by carbaryl. Similarly, catalase, an enzyme that catalyzes the reaction by which hydrogen peroxide is decomposed to water and oxygen, showed increased *activity* in the carbaryl treated buffalo calves. This increase in catalase activity could possibly be explained as an adaptive mechanism by the animal to withstand the oxidative damage caused by hydroperoxides, as explained by the ameliorative effect of catalase treatment upon peroxide accumulation (35).

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Conflict of Interest: None Declared

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