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RESEARCH ARTICLE

CARBENDAZIM TOXICITY IN QUAILS (*CORTUNIX JAPONICA*): PERFORMANCE, CLINICOPATHOLOGIC CHANGES AND EFFECT OF GARLIC

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ABSTRACT

This investigation was undertaken to elucidate the toxic effect of carbendazim CBZ and the role of garlic supplementation in wildlife sentinels. Forty quails were acquired comprising two and four week's old birds (20 per age group). The quails were placed in to 5 different groups (each for 6 & 8 weeks) comprising 4 birds each. Pesticide dosage was 1.25mg/g body weight. 1 gram of garlic was administered per bird. Blood samples were analyzed using the microhaematocrit and cyanmethaemoglobin methods. Cholinesterase (AchE) was quantified as butyryl cholinesterase activity, oxidative stress markers assayed and tissue changes examined microscopically. The quails treated with CBZ were alopecic. There was paralysis in the six week old quail. Poor egg quality was observed in the 8 week old quails Age differences were observed in the haematological parameters. There was increase lipid peroxidation (MDA), demyelination and gliosis in the brain, villi atrophy and cryptal hyperplasia of the intestine. Testicular hypoplasia and disruption of the tubular basement membrane in the testis. These changes were moderately ameliorated by garlic. This study confirms the chronic low dose treatment of Carbendazim in quails.

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INTRODUCTION

Pesticides are very important group of environmental pollutants used in intensive agriculture for protection against diseases and pests. The estimated annual application is more than 4 million tons, but only 1% of this reaches the target pests (Gavrilescu, 2005). Carbamate pesticides are generally short-lived in the environment (usually lasting only days to months instead of years) and, generally, chemical breakdown is accelerated as temperatures or pH or both increase (Rajeswary, 2007, Omonona and Jarikre, 2015). It is important to understand, however, that there is a wide range of toxicity of pesticides and wide variation in cutaneous absorption, making specific identification and management quite important (Bridges, 2000). The toxicity of carbamate pesticides is due to the disruption and inhibition of cholinesterase (ChE) enzymes. These enzymes are involved in transmitting normal nerve impulses throughout the nervous system. An acute pesticide dose reduces the activity of ChEs, and nerve impulses cannot be transmitted normally. This can paralyze the nervous system, and it may lead to death, usually from respiratory failure (Gaspari and Paydarfar 2007).

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Birds appear to be more sensitive than other vertebrates to the toxic effects of Organophosphates OP and possibly carbamate pesticides. More than 100 avian species have been poisoned by these pesticides. Waterfowl, passerines, and raptors are the species most commonly identified in reported OP and carbamate-related mortalities in the United States (Hill, 1995). Raptors and other bird species become victims of secondary poisoning when they scavenge dead animals poisoned by pesticides or when they feed on live animals or invertebrates that are unable to escape predation because of pesticide intoxication. Age, sex, diet, and body condition all are factors that affect a bird's susceptibility to pesticide poisoning. Generally, embryos and young birds, particularly the dependant or altricial birds, appear to be more sensitive to carbamate compounds than adults. Dietary deficiencies, low fat reserves, poor physiological condition, and high energy needs, such as migration or high metabolic rates, may increase vulnerability to these compounds. Behavioural traits may also increase the potential for exposure to carbamate compounds.

Studies have highlighted the importance of research on the effects of amphibian exposure to novel stressors (e.g., pesticides) at levels as low as those found in the field (Sparling *et al*., 2001). Pesticide concentrations detected in the field usually may not cause mortality (Hayes *et al.,* 2002), and

therefore there is need for this ecotoxicological study focusing on sub lethal responses. Pesticide concentrations found in the environment have been shown to have negative effects on growth, development, immune responses, and behavior of tadpoles (Hayes *et al*., 2002; Christin *et al*., 2003; Broomhall, 2005) and ecosystem in extension. Species at increased risk are those that congregate in areas of treated habitats, gorge on a food source (like geese), forage in treated substrates, or feed on target organisms shortly after applications of these compounds. Moreover, some of the potential negative effects of pesticides may become apparent only when present with other environmental stressors, such as predation (Relyea and Mills, 2001; Relyea, 2005).

Common routes of exposure of birds to pesticides include: consumption of treated seeds, vegetation with pesticide residues, dead or struggling poisoned insects, granular formulations as grit, food, or coincidentally with other food items, carrion killed by a pesticide, food intentionally baited with pesticide, live animals intoxicated with pesticide, water contaminated with pesticide from runoff or irrigation, inhalation, and or absorption through the skin. Also, there can be considerable variability in the sensitivity of individual species to these pesticides. The utilization of garlic, balanced and formulated in a suitable dose can decrease mortality rate and increase immunity in locally raised quail (Omonona and Jarikre, 2014). However, the impact of carbendazim on the aviary in our environment and its link to wildlife morbidities has to be elucidated. Therefore, this present investigation was undertaken to elucidate the toxic effect of carbendazim and the role of garlic supplementation in ameliorating this effects in the quail.

MATERIALS AND METHODS

Experimental animals

The experiment was carried out at the domestication unit of the department of Wildlife and Ecotourism Management, University of Ibadan. Research protocol followed ethics for biomedical research. Forty quails were purchased from a local farm. The quails comprise two and four weeks old birds (20 per age group). The quails were fed standardised growers mash. Vitalyte was added to their water for about three (3) days and stabilized for about 3weeks. Afterwards the quails were randomly sorted based on age in to 5 different groups (each for 6 & 8 weeks) comprising 4 birds each (Table 1).

Table 1. Experimental design on Carbendazim toxicity in quails

Group/Age	6	8
I	CBZ +	CBZ +
II	CBZ ++	CBZ ++
III	CBZ & Garlic	CBZ & Garlic
IV	Garlic only	Garlic only
V	Blank	Blank

Pesticide & Garlic Used

The pesticide used was forcelet, a synthetic agricultural fungicide which contains 50% carbendazim, belonging to the toxicity class IV. Pesticide dosage was 1.25mg/g body weight dissolved in the feed. Garlic supplementation was through the use of dry grinded garlic in which 1 gram was administered per bird (Summer foods, Nigeria ltd, IITA).

Sample collection and Analysis

Blood samples were collected from the quails after 6 weeks of exposure. The blood samples were analyzed for haematological parameters including; Packed Cell Volume PCV, Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Platelet count, Haemoglobin (HB) concentration, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), and Mean Cell Volume (MCV), White blood cell (WBC) count and Differential White Blood Cell Count Using Battlement Method.

Hematologic, Cholinesterase, Oxidative assays

The Packed cell volume PCV, Hemoglobin concentration, Red cell count was estimated by the microhaematocrit method, Leucocyte and Platelet counts were determined using the haemocytometric method as described by Schalm (1986). Cholinesterase (AchE) was quantified as Butyryl Cholinesterase activity using an in vitro diagnostic kit (Fortress Diagnostics, BXC0801), following manual procedure. In brief, 1000µl of the working reagent and 20µl of the sample were pipetted into the test tube, mixed and initial absorbance read after 90 seconds incubation at the assay temperature. Similarly, 20µl of the sample was incubated with 1000µl of buffer and 200µl of substrate before reading absorbance activity. Wavelength was set at 405nm, 37^0**C** and 1cm cuvette light path.

Activity= ΔAbs/min X 55000 [serum start]
Activity=ΔAbs/min X 65800 [substrate start]

The determination of Malondialdehyde (MDA) was carried out using the thiobarbituric acid (TBA) test as described by Vidyasagar *et al.* (2004). The concentration of MDA was read from standard calibration curve in nanomoles of MDA per ml of serum.

Concentration of MDA= absorbance at 540nm/1.56x105$m^{-1}cm^{-1}$

Superoxide Dismutase (SOD) activity was estimated using the method of Misra & Fridovich (1972). In brief, 1ml of serum was diluted in 9ml of distilled water. Aliquot of 0.2ml was added to 2.5ml of 0.05M phosphate buffer at pH 10.2 to equilibrate in the spectrophotometer. The reaction was started by the addition of freshly prepared 0.3ml of adrenaline. Reference cuvette contained 2.5ml of phosphate buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance was monitored every 30 second for 150 seconds and expressed in Unit/mg protein.

% Inhibition= increase in absorbance for substrate X 100/increase in absorbance of blank

Catalase (CAT) activity was estimated using method of Sinha (1972). Briefly, dichromate was reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2). Chromic acetate produced was measured colorimetrically at 570 nm. Result was expressed in µmol/mg protein.

Pathology

Samples from the liver, kidney and brain were processed routinely for histopathology. Slides were evaluated using the Olympus light microscope (CX21) attached to a digital computerized camera (AmScope, MU900).

Statistics

Results were expressed as the means ± standard deviation of the values for each haematological parameter in each group for infected quails at 6 weeks old, 8 weeks old and for 8 weeks old after treatment and the value obtained were compared using T-test, AVNOVA and Bonferoni tests at 5% confident interval.

RESULTS

Physical and Behavioural Observation

The quails treated with carbendazim CBZ showed signs of alopecia especially as loss of plumage around the neck and dorsum. There was an apparent case of outright paralysis in the six week old quail exposed to CBZ. Poor egg quality (loss of spots and egg shell thinness) was observed in the 8 week old quails treated with CBZ.

quails treated with CBZ and the differences were statistically significant (P <0.05). The Mean Lymphocyte and Monocyte counts were significant (P<0.05) while the heterophil, eosinophil and basophil counts were insignificant (P>0.05) (Table 2).

In eight weeks old quails

A decrease in mean PCV values was observed between the groups but was significant. (P<0.05). The differences in the Hb, RBC and Platelet counts were statistically insignificant (P>0.05). The WBC was statistically insignificant (P>0.05). Differentially, the Lymphocyte count significant (P<0.05) but the Heterophil and Basophil counts insignificant (P>0.05), Monocytes and Eosinophil counts significant (P<0.05) (Table 2). Comparatively, there was an increase PCV, HB, RBC in quails exposed to garlic only, those that were exposed to CBZ & garlic, and those that were exposed to CBZ. Platelet decreased significantly (p<0.05). The erythrogram values were observed to be lower in the adult quails and while reverse were observed in the leucogram. Garlic supplementation reduced the erythrogram values (group III) although this was insignificant (P>0.05). There was also slight increase in the leucocyte counts.

Table 2. Haematologic parameters in 6 & 8 week old quail birds exposed and non-exposed to lambda-cyhalothrin

Group	I		II		III		IV		V	
	6WK	8WK	6WK	8WK	6WK	8WK	6WK	8WK	6WK	8WK
PCV	46.3±1.4	41.7±1.3	46.5±2.5	36.3± 1.8	44.1±3.0	37.8±6.3	45.5±2.5	40.0±5.0	43.8±2.9	44.2±1.2
HB	14.8±0.7	14.1±0.5	15.8±1.1	12.3±0.6	15.5±2.3	12.5±2.4	14.8±1.2	13.7±2.0	14.8±1.0	13.8±0.6
RBC	3.9±0.2	3.5±0.2	4.4±0.2	3.6±0.1	4.0±0.2	3.9±0.7	4.4±0.2	4.2±0.5	3.8±0.2	3.8±0.2
PLT	15.4±1.7	19±1.6	15.3±1.6	22±1.5	15.1±1.6	14.9±1.5	13.3±2.4	9.3±1.7	12.3±0.4	18±1.9
WBC	18.6±0.6	25.0±1.3	20.1±0.6	24.0±2.1	16.1±1.6	19.2±0.8	14.1±1.0	15.9±1.5	17.3±0.9	19.0±2.7
LYM	12.0±0.5	16.0±1.6	12.7±1.0	17.0±1.9	7.7±1.3	9.0±1.2	7.5±1.9	7.5±1.9	11.2±0.6	11.0±1.6
HET	5.8±0.6	6.6±0.6	6.1±0.8	5.7±0.2	6.6±1.6	8.7±1.5	6.8±1.6	8.2±3.0	4.6±0.7	7.8±1.5
MON	0.8±0.1	1.8±0.7	1.0±0.4	1.1±0.1	1.2±0.8	0..9±0.5	0.8±0.4	0.2±.0.1	1.2±0.8	0.2±0.1
EOS	0.2±0.1	0.5±0.8	0.2±.0.1	0.1±0.0	0.5±0.1	0.7±0.1	0.0±0.0	0.0±0.0	0.2±0.0	0.0±0.0
BAS	0.1±0.1	0.5±0.1	0.1±0.0	0.1±0.0	0.3±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.0±0.0
MCV	120±4.0	120±10	110±11	100±10	110±6.0	110±2.8	110±3.6	110±4.2	110±3.3	120±10
MCHC	32.6±0.6	33.7±0.2	33.8±0.9	33.9±0.2	32.6±0.6	32.6±1.3	34.6±0.6	34.1±0.6	33.6±0.2	31.3±1.6

Packed Cell Volume- PCV (%), Haemoglobin Concentration-Hb (g/dl), Red Blood Cell-RBC (*10^3/ µL), White Blood Cell-WBC (*10^3/ µL), Platelet Count-Plate (*10^5/ µL), Lymphocytes-Lympho, Neutrophils-Neutro, Monocytes-Mono, Eosinophils-Esino, Mean Cell Volume-MCV (fl), Mean Cell Haemoglobin-MCH (%), Mean Cell Haemoglobin Concentration-MCHC (pg)

Table 3. Biochemical parameters in 6 & 8 week old quail birds exposed and non-exposed to Carbendazim

Group	I		II		III		IV		V	
	6WK	8WK	6WK	8WK	6WK	8WK	6WK	8WK	6WK	8WK
CHOLIN	4.54±1.10	4.34±2.0	5.09±0.69	3.77±0.53	5.03±0.94	5.03±0.93	6.47±3.01	6.47±3.01	4.0±1.2	3.42±1.13
MDA	91.03±89	242±341	812±80.4	825±116	237±144	237±143	185±0.3	185±0.3	322±191	144±0.9
SOD	4.45±0.3	4.8±0.4	6.67±0.8	4.53±0.3	6.6±0.9	6.8±0.92	7.8±1.8	7.8±1.8	8.4±0.6	8.52±0.73
CAT	1.94±0.04	2.48±0.6	1.95±0.02	1.91±1.1	1.92±0.01	1.92±0.01	2.01±0.1	2.01±0.1	2.12±0.2	1.91±0.01

AchE (CHOLIN)- Acetylcholinesteerase (U/L); MDA- Malonyl aldehyde (Nmol/mil); SOD- Superoxide dismutase (Unit/mg protein); CAT-Catalase (Unit/mil). Values with different superscripts in row are statistically different and * are significant at 0.05

Haematologic changes

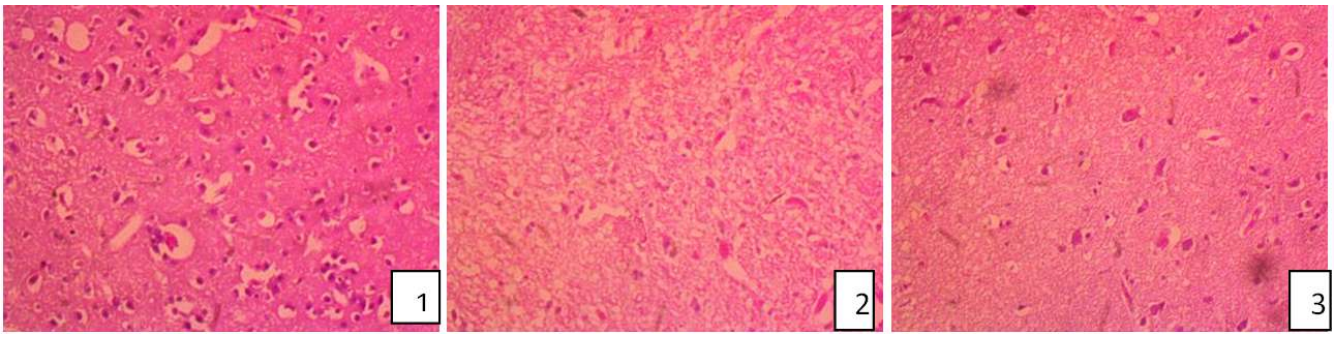
In six weeks old quails

There was no significant difference in the mean PCV, Haemoglobin (Hb) concentration, Red Blood Count (RBC) between the groups (P>0.05). The differences in the Platelet count were also insignificant (P>0.05). The Mean White blood Count (WBC) for Control, CBZ^{+} and CBZ^{++} are 17.3±0.9, 18.6±0.6 and 20.1±0.6 respectively. There was a steady increase in the mean White blood Count (WBC) values in the

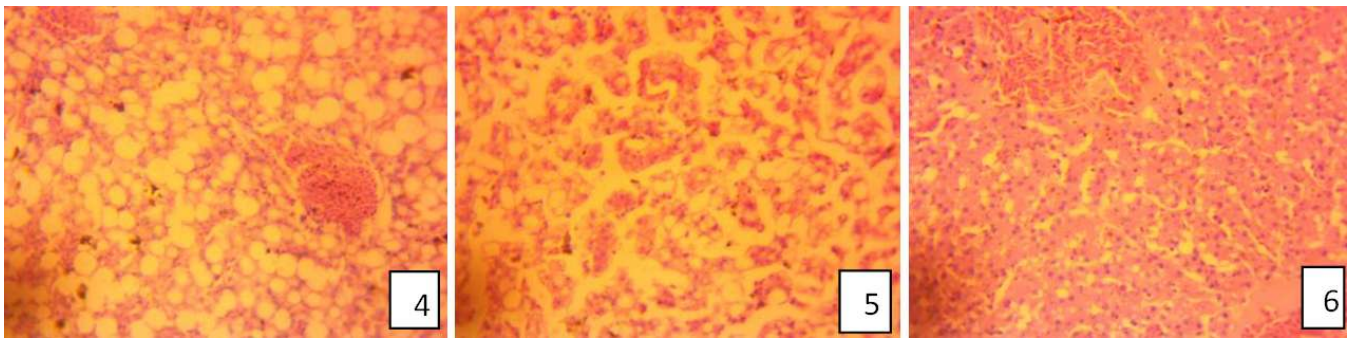
Biochemical assay

The increases in Malondialdehyde (MDA) was significant (p<0.05) in the pesticide treated quails compared to control. Garlic supplementation reduced MDA level significantly (p<0.05). There was no remarkable difference in acetylcholinesterase levels in the pesticide treated quails (p>0.05). Garlic supplementation increased the AchE levels (Table 3). Carbendazim treatment reduced superoxide dismutase (SOD) activity in the exposed quails (p<0.05).

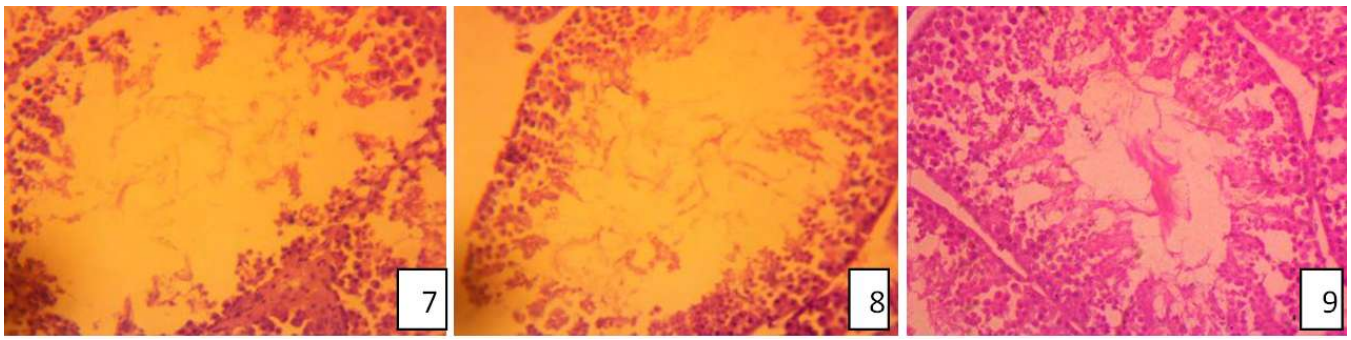
Supplementation with garlic increased SOD activity in the rats ($p<0.05$). Slight differences were observed in Catalase activity.



Photomicrograph of brain; pesticide treated (1) showing demyelination & gliosis. Garlic supplanted group (2). Control (3) showing normal seminiferous tubules. HE x100



Photomicrograph of liver; pesticide treated (4) showing diffuse hepatocellular degeneration. Garlic supplanted group (5). Control (6) showing hepatic cords. HE x100



Photomicrograph of testes; pesticide treated (7) showing spermatogenic arrest. Garlic supplanted group (8). Control (9) showing normal seminiferous tubules. HE x100

Histopathology

Microscopically, there were foci of demyelination, spongiosis and gliosis in the brain (cerebrum) in the CBZ treated quails (Fig. 1-2) but normal in control (Fig. 3). There was vili denudation, atrophy and cryptal hyperplasia of the intestine. Severe diffuse vacuolation of hepatocytes with attenuation of sinusoids in the liver of in CBZ treated quails (Fig. 4-5) but normal in control (Fig. 6). There was spermatogenic arrest, testicular hypoplasia and disruption of the tubular basement membrane in the testis (Fig. 7-8).

DISCUSSION

Agricultural practices affect natural habitats in several ways, such as through land conversion, increased fragmentation, and agrochemical contamination (Hecnar, 1995; Vos and Chardon, 1998).

Many compounds introduced into the environment by human activity are capable of disrupting the endocrine system of animals, including fish, wild life and humans. Among these chemicals are pesticides, industrial chemicals, pharmaceuticals, phytochemicals and other anthropogenic products (Rajeswary *et al*., 2007). Sparling *et al*. (2001) demonstrated increased endocrine activity in amphibians exposed to pesticides in the field explaining declines in some Californian Ranid populations. Relyea (2005) detected significant disruption in species richness in aquatic communities including both secondary positive and direct negative effects in the amphibian community at ecologically relevant pesticide concentrations. Carbendazim (methyl-2-benzimidazole carbamate, MBC) is a systemic broad spectrum fungicide controlling various fungal pathogens. Carbendazim is formed during the degradation of benomyl and thiophante-

methyl. It is also used as a preservative in paint, textile, papermaking, leather industry and warehousing practices, as well as a preservative of fruits (Selmanoglu *et al.,* 2001). Carbendazim is well absorbed (80–85%) after oral exposure and is subsequently metabolized into many compounds within the organisms. The main metabolites are 5-hydroxy- 2-benzimidazole carbamate (5-HBC) and 5, 6-hydroxy-2-benzimidazole carbamate-*N*-oxides (5,6-HOBC-*N*-oxides). Carbendazim is poorly catabolized and remains in tissues such as gonads, liver, adrenals, adipose tissue, skin and other organs (WHO 1993). Apparently, severe toxicity was recorded in the young quails as compared to the adult quails. There was leucopenia, lymphopenia and paralysis (neurotoxicity). Slight anemia was observed in the adult birds, and egg quality and testicular toxicity observed. This may possibly explains the influence of carbendazim on the endocrine and reproductive systems. Similar histologic effects were reported by Hess and Nakai (2000) from male reproductive system in rats and in amphibians (Broomhall, 2005). Testicular pathology develops within hours following carbendazim (CBZ) exposure (Nakai and Hess, 1994; Markelewicz *et al*., 2004).

Malignant lymphomas were induced after 20-43 weeks in mice whose mothers were treated with carbendazim combined with nitrite (Börzsönyl *et al*., 1976). Seiler (1976) demonstrates that the action of carbendazim is on mitosis, by inhibiting the mitotic process, and significant role in the spindle inhibitory action. In a micro-nucleus test however, a dose-related increased number of nucleated erythrocytes was observed. The increase in MDA level observed following oral exposure to CBZ compared with control indicates the oxidative damage resulting from enhanced reactive oxygen species (ROS) production as also observed by Banerjee *et al*. (1999) and Adedara *et al*. (2013). In the present investigation, restoration of normal haematologic and biochemical values in the quails was achieved with garlic supplementation. In conclusion, the present study confirms that chronic low dose treatment of CBZ is capable of inducing reproductive toxicity through increased oxidative stress, by impairing enzymes, antioxidant cellular defense and enhancing the hydrogen peroxide, hydroxyl radicals and lipid per oxidation in the tissues. However, these effects were reversible upon garlic supplementation.

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