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Full Length Research Paper

L-carnitine protects against oxidative stress induced by sublethal exposure to the synthetic pyrethroid, lambda-cyhalothrin, in rats

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Abstract

Synthetic pyrethroids account for more than 30% of insecticides used worldwide in agricultural, domestic and veterinary applications. Although mainly used as insecticides, these compounds have a wider spectrum of action including mammalian toxicity. We conducted experiments to determine physiological and biochemical responses of Wistar rats orally exposed for 30 days, to sublethal doses of lambda-cyhalothrin (1/100th and 1/10th of the LD50). We also investigated the protective role of the antioxidant, L-carnitine. We observed that body weight was adversely affected by exposure to lambda-cyhalothrin, as was the relative weight of testes; weights of liver and spleen, however, were significantly increased. Several haematological variables (total erythrocyte count, haemoglobin, serum protein) were negatively affected while total leukocyte count was increased. Plasma concentrations of glucose and urea were also elevated. Activities of metabolic enzymes (aspartate aminotransferase, alanine aminotransferase, acid phosphatase, alkaline phosphatase), were significantly enhanced in serum but suppressed in liver. Activity of lactate dehydrogenase was also increased in serum, and greatly increased in liver. Acetylcholinesterase activity in serum was not affected but activity of this enzyme in the brain was markedly suppressed. A significant increase was seen for several biochemical indicators of oxidative stress, such as thiobarbiturate-reactive substances in blood and liver and the concentrations of reduced glutathione in liver, testis, brain and kidney. The measurements taken together indicate that lambda-cyhalothrin causes a sublethal, dose-dependent syndrome of oxidative stress, with additional effects on haematopoiesis and carbohydrate metabolism. Most strikingly, simultaneous dosing of the anti-oxidant L-carnitine removed the oxidative stress status and eliminated the harmful effects in almost all cases. Our data confirm that sublethal mammalian toxicity of lambda-cyhalothrin is dominated by oxidative stress, despite the fact that its use as a pesticide relies on neurotoxicity. The anti-oxidant L-carnitine has a strong protective effect against lambda-cyhalothrin toxicity.

Keywords: Lambda-cyhalothrin, L-carnitine, oxidative stress, anti-oxidant, oral exposure, Wistar rat

INTRODUCTION

Synthetic pyrethroid insecticides are analogs of naturally occurring pyrethrins and have been developed as new-generation plant protection products with a short life-time in the environment, high toxicity to specific arthropod pests and low toxicity to birds and mammals. The favorable properties of these insecticides have promoted wide-spread application in virtually all sectors of crop protection and pest control (Kidd and James, 1991; Kale et al., 1999; Sogorb and Vilanova, 2002). They now account for more than 30% of insecticides used world-wide.

The pesticidal action of pyrethroid insecticides is due to neurotoxicity, which is manifested by an increased excitation of the central and peripheral nervous systems (Luty et al., 1998). Traditionally, pyrethroids have been divided into two groups, based on their mode of action. Type I pyrethroids affect sodium channels in nerve membranes and open them for relatively short periods (ms), inducing repetitive neuronal discharges and causing a prolonged negative after-potential. Type II pyrethroids produce a longer delay in sodium channel inactivation leading to a persistent depolarization of the nerve membrane without repetitive discharge. However, evidence from some studies suggests that other target sites, such as calcium and chloride channels, are also involved in the acute toxicity of pyrethroids (Soderlund *et al*., 2002; Hossain et al., 2006; Symington et al., 2007).

Lambda-cyhalothrin is a synthetic pyrethroid insecticide and acaricide acting according to type II. It is used to control a wide range of pests in a variety of agricultural settings. Pests controlled include aphids, colorado beetles and lepidopteran larvae. Crops on which it may be applied include cotton, cereals, hops, ornamental plants, potatoes and various vegetables (Kidd and James, 1991). Cytogenic effects of lambda-cyhalothrin have been reported using endpoints such as micronucleus formation, chromosomal aberrations, and sister chromatid exchange (Fahmy and Abdalla, 2001). In addition, a few studies report the involvement of oxidative stress in pyrethroid-induced toxicity (Giray et al., 2001; El-Demerdash et al., 2004; Prasanthi et al., 2005; Yousef et al., 2006).

Antioxidants provide defence against free radicals and oxidative stress. They act as free radical scavengers and slow down not only radical formation but also the damaging effects of radicals in the body (Nice, 1997). Well-known anti-oxidants are vitamin C, vitamin E, β-carotene, isoflavones, folic acid and L-carnitine (Giray et al., 2001; Yousef et al., 2003, 2006; El-Demerdash et al., 2004).

Carnitine is a vitamin-like substance that is structurally similar to amino acids. Most carnitine is obtained from the diet. It can also be synthesized endogenously by skeletal muscle, heart, liver, kidney and brain from the essential amino acids lysine and methionine (Rebouche and Seim, 1998). Carnitine is a vital component in lipid metabolism; its major biochemical role is supporting the transport of long-chain fatty acids into the mitochondrial matrix for β-oxidation (Aydogdu et al., 2006). It also prevents the accumulation of end-products of lipid peroxidation (Kalaiselvi and Panneerselvam, 1998). Other roles include removal of excess acyl groups, peroxisomal fatty-acid oxidation, and antioxidant activity (Czeczot and Scibior, 2005). Carnitine can also act as a chelator by decreasing the concentration of cytosolic iron, which plays a very important role in free radical chemistry (Rani and Panneerselvam, 2002).

In this research we studied the interaction between sublethal exposure to lambda-cyhalothrin and L-carnitine on the physiological and biochemical level. We argued that if mammalian toxicity of pyrethroids is due to oxidative stress, it should be possible to compensate the effects by simultaneous administration of an anti-oxidant such as L-carnitine. Our experiments were designed to test this hypothesis.

MATERIALS AND METHODS

Chemicals

Formulated lambda-cyhalothrin, a clear to slightly hazy, stable homogeneous liquid (5%), free from visible suspended matter and sediment, and was purchased from Chema Industries, Egypt (www.chema.com.eg). The emulsion was diluted with water to arrive at a concentration suitable for dosing. L-carnitine was supplied by Rexall, Inc., Boca Raton, U.S.A.

Animals and treatment

The experiments were approved by the Ethical Committee for Animal Experiments of the Institute for Medical Research, Alexandria University, from which the rats were also obtained. Male Swiss albino rats (Wistar), 3-4 months of age and with an average body weight of 180-200 g were used in this study. The rats were housed in cages under identical conditions, in the animal house of the Institute of Graduate Studies and Research, Alexandria University. Feed and water were provided *ad libitum* during the experimental period (30 days). The animals were supplied with commercially available dry food pellets. Animals were maintained in a controlled atmosphere of 12 hours dark/light cycle, at an ambient temperature of 22 ± 2 ºC , and 50-70 % air humidity. After a period of acclimation, animals were divided into six groups of five rats each. Each group was housed in a separate cage. The first group was used as a control. The second group was used to study the sole effect of L-carnitine (200 mg/kg b.wt.). The third group was used to study the effect of a low oral dose of lambda-cyhalothrin

(0.79 mg/kg b.wt.; 1/100th of the LD_{50}). The fourth group was used to study the effect of the combination of L-carnitine with a low dose of lambda-cyhalothrin. The fifth group was used to study the effect of a high dose of lambda-cyhalothrin (7.9 mg/kg b.wt., 1/10th of LD_{50}) and the sixth group was used to study the effect of the combination of L-carnitine with a high dose lambda-cyhalothrin. The vehicle used for L-carnitine was corn oil. Animals were treated with the test compounds every day for 30 days. The LD_{50} for lambda-cyhalothrin has been determined as 79 mg/kg for male rats (Kidd and James 1991), while the L-carnitine dose was derived from Arafa et al. (2003). The proper doses of lambda-cyhalothrin and L-carnitine for each animal were applied using a syringe that was inserted orally with the help of a plastic tube into the oesopharyngeal region.

At the end of the experimental period, total body weight of each animal was recorded. The animals were sacrificed by cervical decapitation and dissected. The fresh weights of liver, brain, kidney, lung, heart, spleen, testis and epididymis were recorded (the organs were weighed after blotting them dry). The organs were individually identified and kept frozen (-60°C) until assays were performed on them.

Physiological and biochemical measurements in plasma

Blood samples (a few ml) were collected during dissection and placed immediately on ice. Heparin was used as an anticoagulant. Plasma was obtained by centrifugation of samples at 860 g for 20 min and stored at -60°C until analysis. Non-coagulated blood was tested, shortly after collection, for hemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC) and packed cell volume (PCV). Blood Hb concentration was determined by the cyanomethaemoglobin procedure (Wintrobe, 1965). Erythrocytes were counted using an AO bright line hemocytometer with a light microscope at 40x10 magnification. Blood samples were diluted 200 times by physiological saline (0.9% sodium chloride solution) before counting. Leukocytes were counted using an AO bright line hemocytometer with a light microscope at 10x10 magnification after diluting blood samples 20 times with a diluting fluid (1% acetic acid solution and a little of Leishman's stain) before counting (Hepler, 1966). Micro Wintrobe hematocrit tubes and a hematocrit centrifuge were used to determine the packed cell volume (PCV, or hematocrit value). Blood was centrifuged for 5 min at 4000 rpm and the PCV value was obtained by reading the packed cell volume on the graded hematocrit tubes (Wintrobe, 1965).

Stored plasma samples were analyzed for total protein (TP) by the Biuret method according to Henry et al. (1974). Albumin (A) was determined by the method of Doumas et al. (1977). Globulin (G) was determined as the difference between total protein and albumin. The concentration of glucose was determined with kits from Biosystems, S.A. Costa Brava, 30-Barcelona (Spain). Plasma concentrations of urea and creatinine were determined by the methods of Patton and Crouch (1977) and Henry et al. (1974), respectively. Plasma total bilirubin was measured using the method of Pearlman and Lee (1974). Plasma concentrations of total lipids, cholesterol, and triglycerides (TG) were determined according to the methods of Knight et al. (1972), Watson (1960) and Fossati and Prencipe (1982), resp. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined according to the methods of Warnick et al. (1983) and Bergmeyer (1985), resp. Very low-density lipoprotein (VLDL) was calculated by dividing the values of TG by 5.

Plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine aminotransferase (ALT; EC 2.6.1.2) activities were determined with kits from Sentinel, Switzerland (using the principle of Eugenio 5-20155, Milan-Italy). Acid phosphatase (AcP; EC 3.1.3.2) activity was determined according to Moss (1984). Alkaline phosphatase activity (AlP; EC 3.1.3.1) was determined according to the method of Principato *et al*. (1985). Plasma lactate dehydrogenase (LDH; EC 1.1.1.27) activity was determined by the method of Cabaud and Wroblewski (1958). Acetylcholinesterase (AChE; EC 3.1.1.7) activity was estimated using acetylthiocholine iodide as a substrate according to the method of Ellman et al. (1961). Thiobarbituric acid-reactive substances (TBARS) were measured using the method of Tappel and Zalkin (1959). Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to Misra and Fridovich (1972). Sulfhydryl groups (SH-groups) were measured after reaction with 5,5'-dithiobis(2-nitrobenzoic acid) using the method of Ellman (1959). Glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to Habig et al. (1974), using p-nitrobenzylchloride as a substrate.

Physiological and biochemical measurements in tissues

Brain, liver, kidney, lung and testes were washed using a chilled saline solution after weighing. The tissues were minced and homogenized (10% w/v) in ice-cold 1.15% KCl with 0.01 M sodium-potassium phosphate buffer (pH 7.4) in a Potter-Elvehjem type homogenizer. The homogenates were centrifuged at 10,000 g for 20 min at 4 °C, and the resultant supernatant was used for different enzyme assays and free radicals by the same methods as mentioned before in plasma. Protein contents of liver, kidney, testes, lung and brain were assayed by the method of Lowry et al. (1951).

Data Analysis

Statistical analyses of the results followed Steel and

Torrie (1981). All variables were subjected to one-way analysis of variance (ANOVA), while the effect of treatments was judged by the F-test at the 5% significance level. Multiple comparisons of means was done by means of Duncan's Multiple Range Test (SAS, 1986).

RESULTS

Body weight and organ weights

All rats survived the 30 days exposure period. Only rats orally administered with a high oral dose ($1/10^{th}$ of LD50) of lambda-cyhalothrin showed a significant decrease (ANOVA, Duncan's test, $p < 0.05$) in body weight in comparison to the control (figure 1). Treatments in which lambda-cyhalothrin was combined with L-carnitine resulted in a body weight higher than the groups treated with lambda-cyhalothrin only, while L-carnitine alone did not cause any change in body weight (figure 1).

The relative weights of testis and epididymis were significantly decreased by lamda-cyhalothrin exposure ($p < 0.05$), while the relative weights of liver and spleen were significantly increased ($p < 0.05$); this also held for kidney (not shown here). The relative weights of brain, lung and heart were not affected by the treatments. The administration of L-carnitine with lambda-cyhalothrin in either low or high doses alleviated the harmful effects on the relative weights of liver, kidney, testes and epididymis (no significant difference with the control group). The effect of the high dose on spleen weight, however, was only partly compensated (figure 1). L-carnitine itself did not cause any changes in the relative weights of all tested organs.

Haematology and plasma variables

Changes in haematological parameters of male rats treated with low and high doses of lambda-cyhalothrin, L-carnitine, and their combinations, are shown in table 1. Statistical analysis revealed that the treatments with both doses of lambda-cyhalothrin resulted in a significant ($p < 0.05$) decline in total erythrocyte count (TEC), hemoglobine (Hb) and packed cell volume (PCV) compared to the control group. The high dose of lambda-cyhalothrin significantly increased total leukocyte count (TLC). L-carnitine alone did not cause significant changes in these parameters, but treatment with the combination of lambda-cyhalothrin in either low or high doses with L-carnitine caused a significant increase of TEC, Hb and PCV compared with the lambda-cyhalothrin groups, and a significant decrease for TLC. The protective effect of L-carnitine was most pronounced for TLC (low dose) and TEC (low and high dose), while partial compensation was achieved for the other variables.

Plasma total protein (TP) and globulin (G) levels were significantly decreased by both the low and the high dose of lambda-cyhalothrin ($p < 0.05$), whereas the A/G ratio showed a significant increase ($p < 0.05$) compared to the control (table 2). The albumin concentration in plasma was not affected by the treatment. The combination of L-carnitine with lambda-cyhalothrin in either low or high doses restored the level of plasma total protein to the control values and it alleviated the toxic effect of lambda-cyhalothrin on globulin and the A/G ratio.

Both doses of lambda-cyhalothrin caused a significant increase ($p < 0.05$) in the levels of plasma glucose and urea, compared to the control group, while only the high dose significantly increased ($p < 0.05$) the concentrations of creatinine and bilirubin (table 3). Treatment with L-carnitine alone had no effect on these variables. Rats treated with lambda-cyhalothrin in either low or high doses in the presence of L-carnitine showed a significant decrease ($p < 0.05$) in plasma glucose, urea, creatinine and bilirubin, as compared with the lambda-cyhalothrin groups, meaning that L-carnitine minimized the toxic effect of lambda-cyhalothrin.

Table 4 shows that both dosings of lambda-cyhalothrin caused a significant ($p < 0.05$) increase in plasma total lipids and cholesterol levels. Only the high dose of lambda-cyhalothrin significantly increased triglycerid concentrations in plasma. The treatment with L-carnitine alone did not cause any changes in the concentration of total lipids, cholesterol and triglycerids, but rats treated with low or high doses of lambda-cyhalothrin in the presence of L-carnitine showed a significantly ($p < 0.05$) lower concentration of plasma total lipids, cholesterol and triglycerides compared to the lambda-cyhalothrin groups.

Concentrations of plasma high density lipoproteins (HDL), low-density lipoproteins (LDL) and very low density lipoproteins (VLDL), were also significantly affected by the treatments. Both doses of lambda-cyhalothrin caused a significant decrease of HDL and a significant increase of LDL. Only the high dose of lambda-cyhalothrin significantly increased VLDL ($p < 0.05$, table 4). Animals treated with the combination of lambda-cyhalothrin, in low or high doses, with L-carnitine, showed a significant decrease ($p < 0.05$) in the concentration of LDL in plasma as compared with the lambda-cyhalothrin groups. Carnitine also decreased to some extent the harmful effect of lambda-cyhalothrin on HDL and VLDL but the means did not reach the control levels (table 4).

Enzyme activities in plasma and organs

Data on activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (AcP), and alkaline phosphatase (ALP) in plasma are shown in table 5. All these activities were significantly ($p < 0.05$

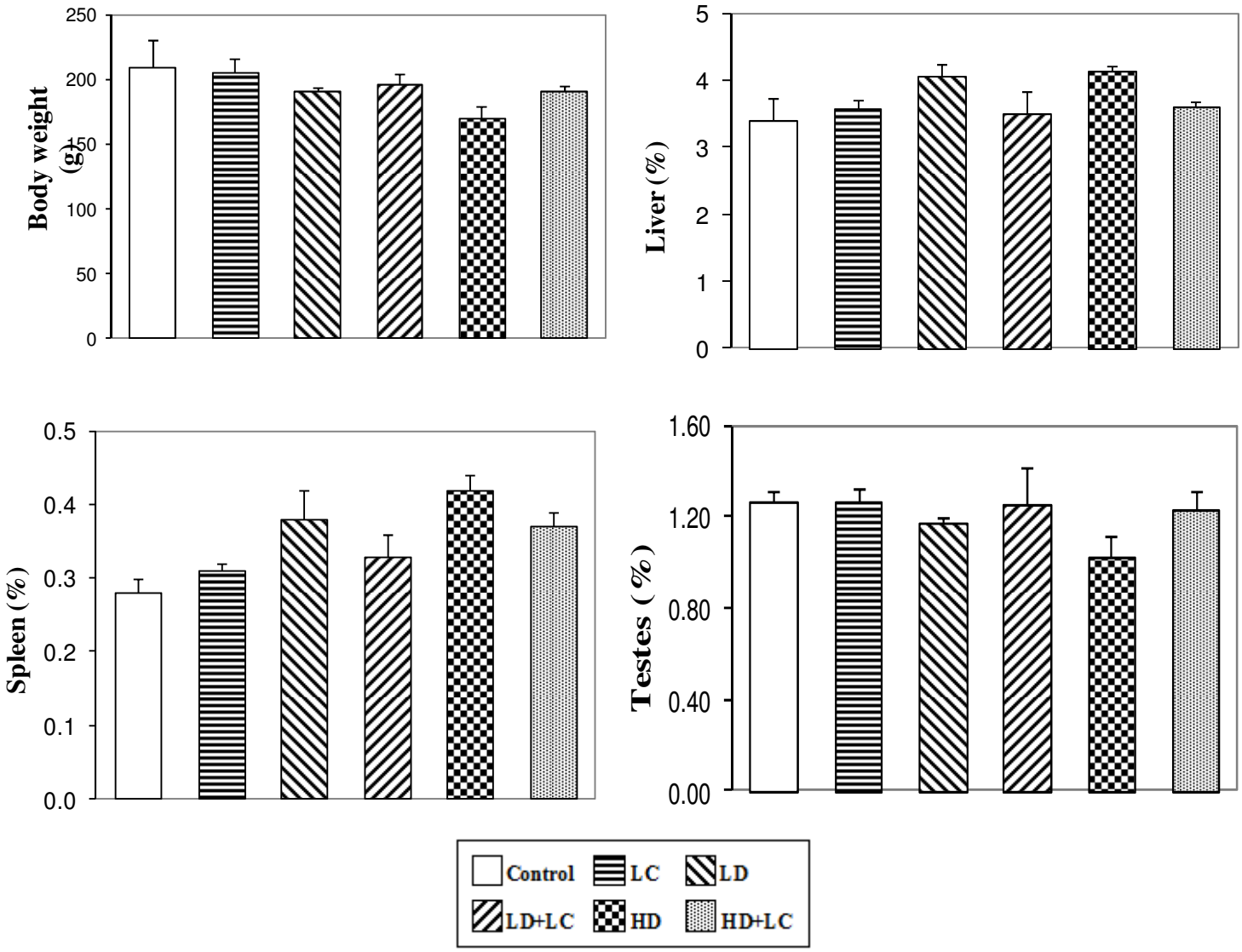

Figure 1. Body weight and relative organs weight of Liver, Spleen and Testes in male rats treated with lamda-cyhalothrin, L-carnitine and their combination.

Table 1. Total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin (Hb) and packed cell volume (PCV) in blood of male rats treated with lambda-cyhalothrin (two doses) and L-carnitine.

	TEC (x 10^6)	TLC (x 10^3)	Hb (mg/dL)	PCV (%)
Control	$7.77^{a,b} \pm 0.7$	$6.48^{b} \pm 1.17$	$15.72^{a} \pm 1.19$	$49.9^{a} \pm 0.8$
L-carnitine alone	$7.56^{a,b} \pm 0.7$	$6.52^{b} \pm 0.96$	$15.22^{a,b} \pm 0.15$	$47.4^{a,b} \pm 1.1$
λ-Cyhalothrin low dose	$5.10^{c} \pm 0.68$	$8.76^{b} \pm 1.16$	$12.26^{c} \pm 0.44$	$42.1^{d} \pm 0.5$
λ-Cyh. low + L-carnitine	$6.06^{b,c} \pm 0.15$	$6.48^{b} \pm 0.74$	$13.80^{b,c} \pm 0.27$	$45.1^{b,c} \pm 1.1$
λ-Cyhalothrin high dose	$3.10^{d} \pm 0.38$	$12.52^{a} \pm 0.54$	$7.78^{d} \pm 0.19$	$36.4^{c} \pm 0.5$
λ-Cyh. high + L-carnitine	$5.82^{c} \pm 0.24$	$9.07^{b} \pm 1.37$	$13.82^{b,c} \pm 0.81$	$43.6^{c,d} \pm 1.3$

Values are expressed as means with their standard errors, over five rats per treatment. Means followed by different superscripts (a, b, c, d) in the same column differ significantly from each other (Duncan's multiple range test test, $p < 0.05$).

Table 2. Plasma total protein, albumin, globulin and albumin/globulin ratio (A/G) of male rats treated with lambda-cyhalothrin (two doses) and L-carnitine.

	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G
Control	$8.97^{a} \pm 0.32$	$4.79^{a,b} \pm 0.10$	$4.18^{a} \pm 1.19$	$1.19^{b} \pm 0.08$
L-carnitine alone	$8.96^{a} \pm 0.59$	$4.53^{b} \pm 0.08$	$4.31^{a} \pm 0.15$	$1.31^{b} \pm 0.43$
λ-Cyhalothrin low dose	$7.09^{b} \pm 0.22$	$4.75^{a,b} \pm 0.14$	$2.34^{b} \pm 0.44$	$2.48^{a} \pm 0.28$
λ-Cyh. low + L-carnitine	$8.48^{a} \pm 0.22$	$4.89^{a} \pm 0.14$	$3.60^{a} \pm 0.27$	$1.40^{b} \pm 0.10$
λ-Cyhalothrin high dose	$6.28^{b} \pm 0.17$	$4.55^{b} \pm 0.08$	$1.72^{b} \pm 0.19$	$2.77^{a} \pm 0.22$
λ-Cyh. high + L-carnitine	$8.11^{a} \pm 0.16$	$4.60^{b} \pm 0.05$	$3.52^{a} \pm 0.80$	$1.33^{a} \pm 0.06$

Values are expressed as means with their standard errors, over five rats per treatment. Means followed by different superscripts (a, b, c, d) in the same column differ significantly from each other (Duncan's multiple range test, $p < 0.05$).

Table 3. Glucose, urea, bilirubin and creatinine concentrations in plasma of male rats treated with lambda-cyhalothrin (two doses) and L-carnitine.

	Glucose (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Bilirubin (mg/dL)
Control	$83^{d} \pm 4.1$	$50^{d} \pm 1.8$	$0.62^{b,c} \pm 0.04$	$0.45^{b} \pm 0.04$
L-carnitine alone	$86^{d} \pm 1.3$	$52^{d} \pm 3.9$	$0.63^{b,c} \pm 0.04$	$0.42^{b} \pm 0.03$
λ-Cyhalothrin low dose	$118^{a,b} \pm 7.0$	$87^{b} \pm 4.7$	$0.73^{a,b} \pm 0.03$	$0.56^{a,b} \pm 0.05$
λ-Cyh. low + L-carnitine	$100^{c} \pm 3.9$	$57^{c,d} \pm 2.2$	$0.59^{c} \pm 0.07$	$0.43^{b} \pm 0.07$
λ-Cyhalothrin high dose	$130^{a} \pm 4.6$	$107^{a} \pm 11.0$	$0.78^{a} \pm 0.03$	$0.63^{a} \pm 0.06$
λ-Cyh. high + L-carnitine	$111^{b,c} \pm 5.0$	$69^{c} \pm 2.1$	$0.64^{a,b,c} \pm 0.06$	$0.45^{b} \pm 0.04$

Values are expressed as means with their standard errors, over five rats per treatment. Means followed by different superscripts (a, b, c, d) in the same column differ significantly from each other (Duncan's multiple range test, $p < 0.05$).

Table 4. Plasma total lipid and lipoprotein profile of male rats treated with lambda-cyhalothrin (two doses) and L-carnitine.

	TL (mg/dL)	Chol. (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	$458^{c} \pm 26.6$	$120^{c} \pm 5.60$	$164^{b,c} \pm 8.85$	$52^{a} \pm 6.13$	$35^{c} \pm 2.56$	$33^{b,c} \pm 1.68$
L-carnitine alone	$459^{c} \pm 38.3$	$117^{c} \pm 3.35$	$161^{c} \pm 4.77$	$51^{a,b} \pm 6.74$	$34^{c} \pm 4.64$	$32^{c} \pm 1.57$
λ-Cyhalothrin low dose	$579^{a,b} \pm 31.1$	$136^{a,b} \pm 7.99$	$181^{b} \pm 1.29$	$37^{b,c} \pm 3.21$	$54^{a,b} \pm 3.43$	$36^{a} \pm 0.24$
λ-Cyh. low + L-carnitine	$489^{c} \pm 32.1$	$120^{c} \pm 3.26$	$168^{b,c} \pm 3.50$	$45^{a,b,c} \pm 4.49$	$42^{c} \pm 4.33$	$34^{b,c} \pm 0.63$
λ-Cyhalothrin high dose	$640^{a} \pm 28.8$	$142^{a} \pm 2.12$	$225^{a} \pm 7.43$	$34^{c} \pm 3.70$	$62^{a} \pm 4.15$	$45^{a} \pm 1.62$
λ-Cyh. high + L-carnitine	$514^{b,c} \pm 25.0$	$122^{b,c} \pm 8.93$	$172^{b,c} \pm 4.20$	$43^{a,b,c} \pm 4.10$	$44^{b,c} \pm 4.18$	$34^{b,c} \pm 0.73$

TL = total lipids, Chol = Cholesterol, TG = Triglycerides, HDL = High density lipoprotein, LDL = Low density lipoprotein, VLDL = Very low density lipoprotein.

Values are expressed as means with their standard errors, over five rats per treatment. Means followed by different superscripts (a, b, c, d) in the same column do not differ significantly from each other (Duncan's multiple range test, $p < 0.05$).

Table 5. Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) in plasma of male rats treated with low or high doses of lambda-cyhalothrin, with or without L-carnitine.

	AST (U/L)	ALT (U/L)	ACP (U/L	ALP (U/L)	LDH (U/L)
Control	$54^{c} \pm 1.2$	$22.5^{d} \pm 1.91$	$5.49^{c,d} \pm 0.51$	$283^{c} \pm 6.4$	$907^{c} \pm 31.1$
L-carnitine alone	$54^{c} \pm 0.8$	$22.8^{d} \pm 0.42$	$5.50^{d} \pm 0.21$	$282^{c} \pm 9.5$	$947^{c} \pm 23.1$
λ-Cyhalothrin low dose	$65^{b} \pm 0.9$	$42.3^{b} \pm 1.20$	$6.72^{b} \pm 0.06$	$326^{b} \pm 14.3$	$1190^{b} \pm 50.5$
λ-Cyh. low + L-carnitine	$56^{c} \pm 1.4$	$24.2^{d} \pm 1.09$	$5.75^{c,d} \pm 0.28$	$294^{b,c} \pm 14.6$	$955^{c} \pm 42.4$
λ-Cyhalothrin high dose	$73^{a} \pm 3.3$	$50.0^{a} \pm 1.57$	$7.58^{a} \pm 0.26$	$365^{a} \pm 17.8$	$1554^{a} \pm 142$
λ-Cyh. high + L-carnitine	$59^{c} \pm 2.0$	$30.8^{c} \pm 1.11$	$5.99^{c} \pm 0.14$	$264^{c} \pm 9.1$	$1036^{b,c} \pm 105$

Activities are measured in U/L, where 1 U is equivalent to the conversion of 1 µmole of substrate per minute. Values are expressed as means with their standard errors, over five rats per treatment. Means followed by different superscripts (a, b, c, d) in the same column differ significantly from each other (Duncan's multiple range test test, $p < 0.05$).

Table 6. Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) in liver of male rats treated with low or high doses of lambda-cyhalothrin, with or without L-carnitine.

	AST (U/mg)	ALT (U/mg)	ACP (U/mg)	ALP (U/mg)	LDH (U/mg)
Control	$118^{a} \pm 2.0$	$154^{a} \pm 0.85$	$12.1^{a} \pm 0.33$	$356^{a} \pm 16.2$	$2.37^{c} \pm 0.14$
L-carnitine alone	$118^{a} \pm 1.6$	$154^{a} \pm 0.82$	$12.2^{a} \pm 0.18$	$353^{a} \pm 9.58$	$2.22^{c} \pm 0.25$
λ-Cyhalothrin low dose	$105^{b} \pm 1.5$	$125^{c} \pm 3.06$	$9.39^{b} \pm 0.24$	$272^{b} \pm 15.1$	$11.93^{b} \pm 1.28$
λ-Cyh. low + L-carnitine	$117^{a} \pm 2.0$	$151^{a,b} \pm 1.16$	$11.8^{a} \pm 0.34$	$349^{a} \pm 5.69$	$2.64^{c} \pm 0.13$
λ-Cyhalothrin high dose	$94^{c} \pm 1.6$	$104^{d} \pm 1.55$	$8.14^{c} \pm 0.13$	$231^{c} \pm 8.52$	$18.36^{a} \pm 1.31$
λ-Cyh. high + L-carnitine	$115^{a} \pm 2.5$	$149^{b} \pm 0.79$	$11.3^{a} \pm 0.21$	$338^{a} \pm 18.7$	$2.95^{c} \pm 0.50$

Activities are measured in U per mg of protein, where 1 U is equivalent to the conversion of 1 µmole of substrate per minute. Values are expressed as means with their standard errors, over five rats per treatment. Means followed by different superscripts (a, b, c, d) in the same column differ significantly from each other (Duncan's multiple range test test, $p < 0.05$).

and $p < 0.01$) enhanced by both doses of lambda-cyhalothrin, while the activities of the same enzymes in liver were significantly ($p < 0.05$) suppressed (table 6). Activity of lactate deydrogenase (LDH) in plasma was also increased by lambda-cyhalothrin (table 5), but in contrast to the other enzymes, its activity in liver was not suppressed but increased, even more than a factor of 5 (table 6). Co-administration of L-carnitine with low or high doses of lambda-cyhalothrin counteracted the effects for all enzymatic activities, except for ALT in high-dosed rats, where a significant difference with the controls remained, implying partial compensation (tables 5,6).

There was no significant effect of the treatments on plasma acetylcholinesterase (AChE) but brain AChE was significantly ($p < 0.01$) decreased, down to 54% of its control value in the low dose and only 32% in the high dose treatment (table 7). L-carnitine added to both the low and the high dose of lambda-cyhalothrin completely restored AChE activity in the brain (table 7).

Table 7. Activity of acetylcholinesterase (AChE) in plasma and brain of male rats treated with lambda-cyhalothrin (two doses), with or without L-carnitine.

	AChE in plasma (U per mL serum)	AChE in brain (U per mg protein)
Control	3.80[a] ± 0.34	13.75[a] ± 0.81
L-carnitine alone	3.93[a] ± 0.34	13.20[a] ± 0.76
λ-Cyhalothrin low dose	3.23[a] ± 0.28	7.47[b] ± 0.54
λ-Cyh. low + L-carnitine	3.80[a] ± 0.53	12.52[a] ± 0.71
λ-Cyhalothrin high dose	2.85[a] ± 0.42	4.40[c] ± 0.76
λ-Cyh. high + L-carnitine	3.67[a] ± 0.48	12.1[a] ± 1.09

Activity is measured in U where 1 U equals 1 µmole of substrate converted per min. Values are expressed as means with their standard errors, over five rats per treatment. Means followed by different superscripts (a, b, c, d) in the same column differ significantly from each other (Duncan's multiple range test, $p < 0.05$).

Anti-oxidant enzymes and biochemical indicators of oxidative stress

The concentration of thiobarbituric acid-reactive substances (TBARS) in plasma was significantly ($p < 0.05$) increased by treatment with both low and high doses of lambda-cyhalothrin. Administration of L-carnitine alone had no effect on both plasma and liver TBARS, however, the addition of L-carnitine to low or high doses of lambda-cyhalothrin significantly ($p < 0.05$) decreased the elevation of TBARS in both plasma and liver. Figure 2 illustrates the effects of the treatments on TBARS in plasma and liver. Similar patterns were observed for testes, kidney, brain and lung (data not shown for sake of brevity).

The activity of superoxide dismutase (SOD) was significantly decreased ($p < 0.05$) in liver of lambda-cyhalothrin-treated rats, and the effect was strongest in the highest dose (table 8). L-carnitine compensated this effect in the low dose (no significant difference with the control) but provided only partial compensation in the high dose, as the activity was still significantly lower than the control value.

There was a slight decrease of activity of glutathione-S-transferase (GST) in liver in lambda-cyhalothrin-treated rats, but this was not significant (table 8). Likewise, L-carnitine did not affect GST activity. A significant ($p < 0.05$) decrease in the levels of reduced glutathione (GSH) was observed in liver, in the low-dosed rats, while the high dose of lambda-cyhalothrin nearly halved the concentration. These effects were completely compensated by simultaneous dosing with L-carnitine (table 8). A similar pattern was seen for the tissue concentration of sulfhydryl (SH) groups, except that in this case L-carnitine alone had a slight, but significant ($p < 0.05$) positive effect on the concentration of SH groups (table 8).

The various biochemical indicators of oxidative stress were not only measured in liver but also in plasma, testis, kidney, lung and brain. These data (not shown here for sake of brevity) confirmed the trend seen in liver: clear evidence of oxidative stress induced by lambda-cyhalothrin, compensated, although not always completely in the high dose, by L-carnitine. To illustrate the systemic nature of oxidative stress induced by lambda-cyhalothrin, and the ameliorating effects of L-carnitine, figure 3 shows the concentration of reduced glutathione in liver, testis, brain and kidney in the various treatments.

DISCUSSION

Our study clearly demonstrated that adverse effects of the insecticide lambda-cyhalothrin occur at dosages considerably below acute oral toxicity ($1/100^{th}$ and $1/10^{th}$ of the LD50). The effects observed are indicative of severe oxidative stress in plasma and several organs of the rats. At the highest dose, body growth was also significantly affected, but there was no mortality. No indications were obtained that lambda-cyhalothrin was neurotoxic to rats. The neurotoxic mode of action that underlies the use of synthetic pyrethoids as insecticides does not seem to be relevant to mammals.

Lambda-cyhalothrin is a type II pyrethroid which has an α-cyano moiety; oxidative stress may be due to the release of cyanohydrins, which are unstable under physiological conditions and further decompose to

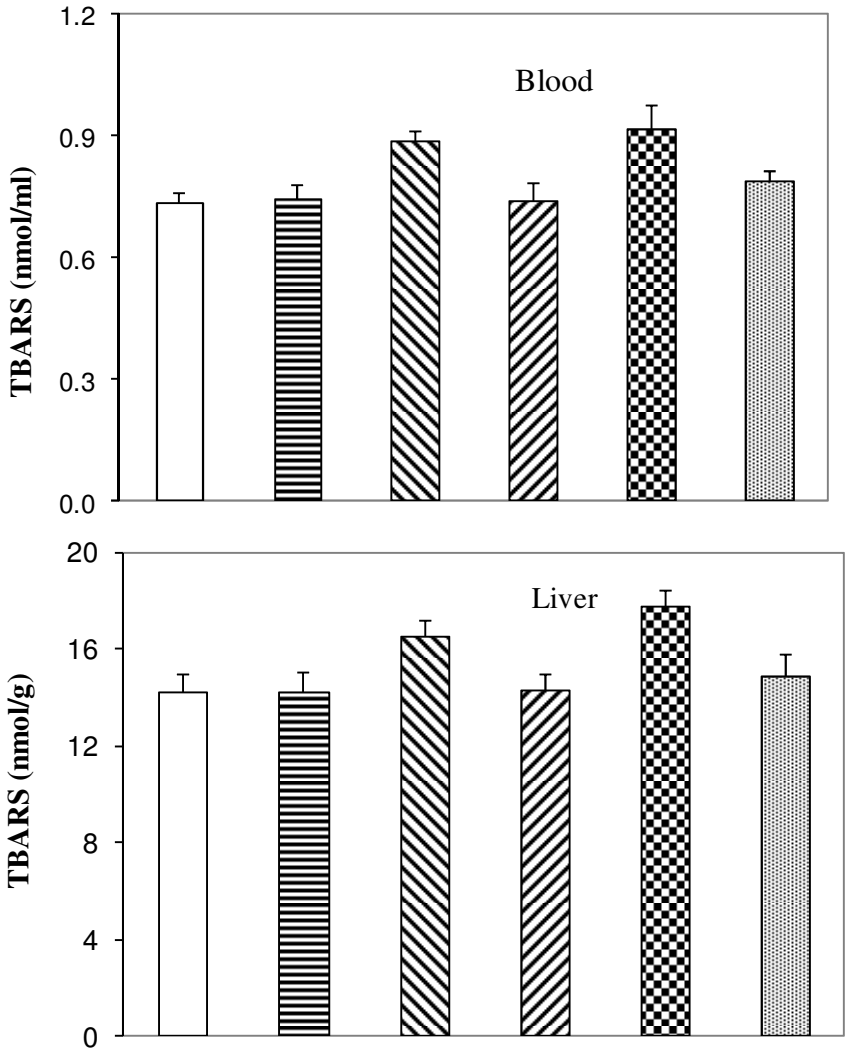

Figure 2. Concentrations of thiobarbituric acid-reactive substances (TBARS) in blood and liver of male rats treated with lambda-cyhalothrin (low dose: 1/100th of LD50, high dose: 1/10th of LD50), with or without L-carnitine: Control, L-carnitine only, Low dose, Low dose with L-carnitine, High dose, High dose with L-carnitine. Means are shown with their standard errors.

cyanides and aldehydes (World Health Organization, 1996), which in turn could act as a source of free radicals (El-Demerdash, 2007). The increased levels of thiobarbituric acid-reactive substances (TBARS) that we observed in plasma and liver are indicative of lipid peroxidation, i.e. membrane damage due to reactive oxygen species induced by lambda-cyhalothrin (cf. Nasuti et al. 2003; Maiti and Kar, 1997; Kale et al. 1999).

In addition to a decrease of body weight, lambda-cyhalothrin also caused an increase of liver weight and a marked increased in the weight of the spleen. The effect on liver weight may be due to proliferation of hepatic cytochrome P450 monooxygenases in the smooth endoplasmic reticulum (Anadón et al. 1991, 1995). The liver was found to accumulate a high concentration of metabolites since it is the major site of pyrethroid metabolism (Rickard and Brodie, 1985). Similarly, Prasanthi et al. (2005) observed significant hypertrophy of the liver when feeding rats with fenvalerate-dosed food. The increase of relative liver weight in our experiment is in line with these data.

The increase of spleen weight may be due to a toxic action of the pesticide on the haemopoietic system or to a direct destructive effect on red blood cells (RBCs), as

Table 8. Activities in liver of superoxide dismutase (SOD, glutathione-S-transferase (GST), concentrations of reduced glutathione (GSH) and concentrations of sulfhydryl groups (SH) for male rats treated with lambda-cyhalothrin (two doses), with or without co-administration of L-carnitine.

	SOD (µmole/min per g tissue)	GST (µmole/h per mg protein)	GSH (mg per g tissue)	SH (mmoles per g tissue)
Control	$85.6^{a} \pm 1.01$	$1.20^{a,b} \pm 0.01$	$14.5^{a} \pm 0.86$	$1.83^{a} \pm 002$
L-carnitine alone	$86.2^{a} \pm 2.76$	$1.23^{a} \pm 0.03$	$14.0^{a} \pm 0.88$	$1.76^{b} \pm 0.03$
λ-Cyhalothrin low dose	$75.9^{c} \pm 1.03$	$1.09^{a,b} \pm 0.02$	$11.7^{b} \pm 0.58$	$1.59^{c} \pm 0.02$
λ-Cyh. low + L-carnitine	$83.1^{a,b} \pm 0.98$	$1.18^{a,b} \pm 0.01$	$14.2^{a} \pm 0.32$	$1.76^{b} \pm 0.01$
λ-Cyhalothrin high dose	$71.5^{d} \pm 0.61$	$1.07^{b} \pm 0.02$	$7.92^{c} \pm 0.39$	$1.45^{d} \pm 0.01$
λ-Cyh. high + L-carnitine	$80.5^{b} \pm 0.57$	$1.14^{a,b} \pm 0.02$	$13.9^{a} \pm 0.75$	$1.71^{b} \pm 0.01$

Values are expressed as means with their standard errors, over five rats per treatment. Means followed by different superscripts (a, b, c, d) in the same column differ significantly from each other (Duncan's multiple range test, $p < 0.05$).

observed by Satyanarayan et al. (2004), Shakoori et al. (1990) and Sayim et al. (2005). Haratym-Maj (2002) observed anaemia in female rats following poisoning with a high dose of deltamethrin and cypermethrin. Dys-erythropoiesis has been postulated as one of the etiological aspects accounting for the anaemic status observed during exposure to different types of pesticides (Keller et al., 2004). Chhabra et al. (1990) also suggested that spleen enlargement may be due to excessive deposition of damaged erythrocytes, in their case caused by aromatic amine toxicity. Similarly, in our case splenomegaly could be a secondary effect of pyrethroid toxicity to the haemopoietic system.

One of the strongest effects of lambda-cyhalothrin seen in our study was on blood glucose. Hyperglycaemia was noted as an effect of pesticide treatment by Yousef et al. (2003), El-Demerdash et al. (2004) and Pournourmohammadi et al. (2007). The marked change in blood glucose level following pesticide treatment is indicative of disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen. Blood glucose elevation is one of the pronounced biochemical changes accompanying pesticide toxicity (Boll et al., 1998; Seifert, 2001; Luskova et al*.,* 2002). We suggest that the exposure of animals to pyrethroids compounds causes a hormonally mediated elevation of glucose level. A variety of toxicants stimulate the secretion of adrenal hormone, resulting in increased levels of circulating adrenocorticotropic hormone which stimulates glycogen hydrolysis (Raja et al., 1992).

Treatment with lambda-cyhalothrin caused a significant increase not only of glucose, but also of plasma urea and creatinine levels. The elevation in blood creatinine and urea is considered a specific and sensitive indicator of impaired kidney function caused by oxidative stress. Janardhan et al. (1988) observed an increase in blood urea which was closely correlated with histopathological degenerative changes in the kidney, and these changes caused disturbance in the transport system of biochemical constituents. Low clearance values for creatinine and urea indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine (Cameron, 1996).

In accordance with our data, disruption of lipid metabolism, including hyperlipidaemia, hypercholesterolaemia and hypertriglyceridaemia is often observed in association with liver damage in rats (Deters *et al*., 2002). An increase of serum triglycerides in rats treated with pesticides has been found in many investigations (Sadurska and Boguszewski, 1993; Gupta et al., 1994.)

The lambda-cyhalothrin-induced lipid peroxidation in liver was accompanied by a marked reduction in reduced glutathione (GSH) levels. GSH acts as a first-line defence against reactive oxygen species. Its depletion to nearly one half of the control level, observed in the high-dose of lambda-cyhalothrin, is another indication of severe oxidative stress induced by this insecticide (cf. Prasanthi *et al*., 2005). A similar explanation was given by Giray *et al*. (2001) who found that a single high dose of cypermethrin increased the oxidatively damaged end-products of lipids in cerebral and hepatic tissue.

In relation to the oxidative stress effects caused by lambda-cyhalothrin, our study has clearly demonstrated an antagonistic effect of the antioxidant L-carnitine. The toxicity of lambda-cyhalothrin on almost all health parameters was completely or nearly completely compensated by simultaneous exposure to L-carnitine, especially at the low dose of the pyrethroid. L-carnitine is a natural substance in various human diets, which is

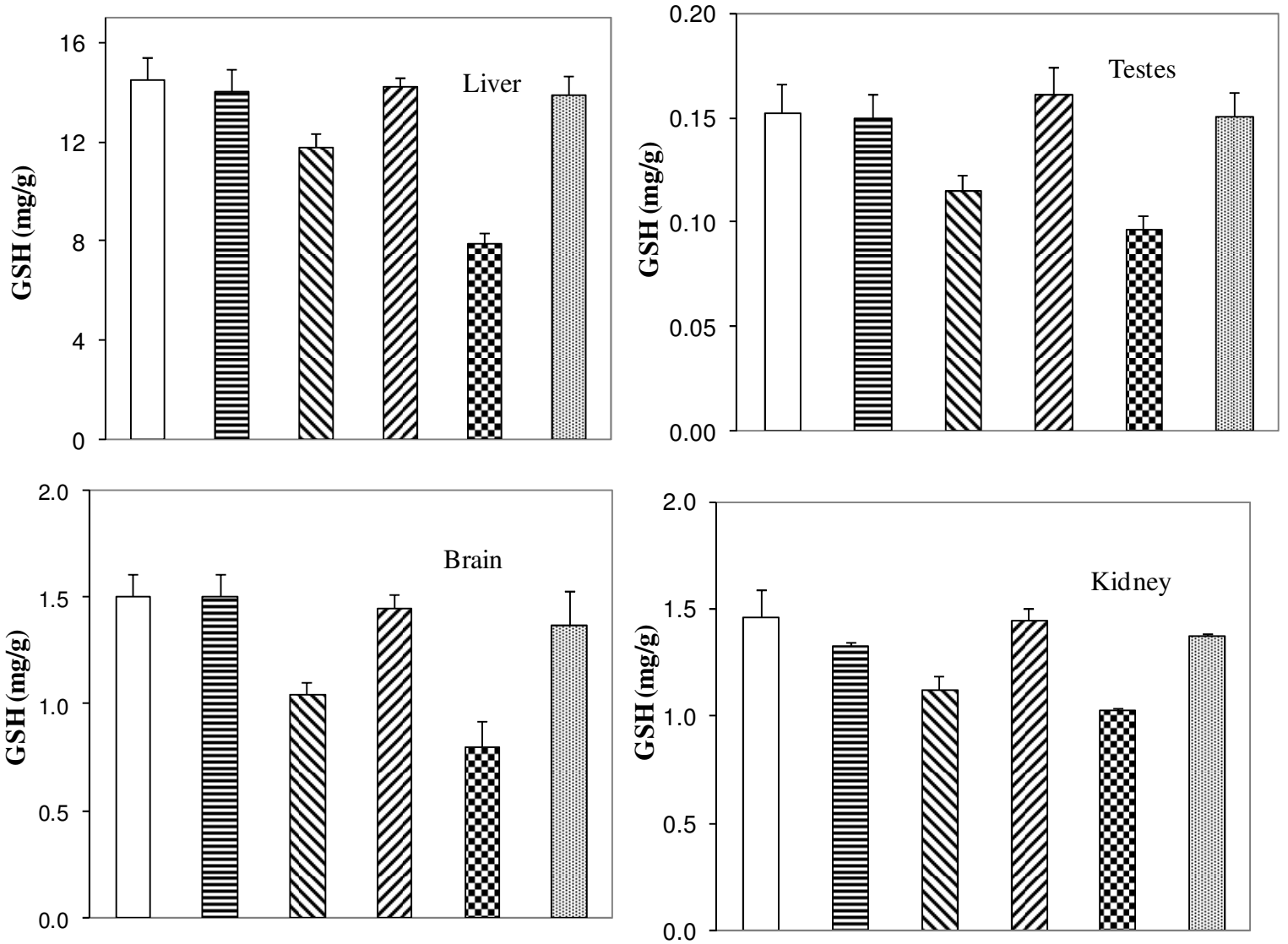

Figure 3. Concentrations of reduced glutathione (GSH) in liver, testis, brain and kidney of male rats treated with lambda-cyhalothrin (low dose: 1/100th of LD50, high dose: 1/10th of LD50), with or without L-carnitine: Control, L-carnitine only, Low dose, Low dose with L carnitine, High dose, High dose with L-carnitine. Means are shown with their standard errors.

considered beneficial in many aspects. Our study illustrates the great importance of nutritional status and dietary factors in pesticide toxicity studies.

L-carnitine has also proven its beneficial effects in other physiological contexts. For example, Bucioli et al. (2012) showed that L-carnitine supplementation could alleviate oxidative stress induced in kidneys of rats subjected to exhaustive exercise. Our study reinforces the conclusion drawn in the biomedical literature that L-carnitine is a powerful antioxidant therapeutic agent.

In conclusion, we have shown that sublethal mammalian toxicity of the synthetic pyrethroid lambda-cyhalothrin is dominated by oxidative stress, not by neurotoxicity, which is the main mode of action in insects and other arthropods. In addition, the anti-oxidant compound L-carnitine is a highly effective antagonist of pyrethroid-induced oxidative stress.

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REFERENCES

Anadón A, Martinez-Larranaga MR, Diaz MJ, Bringas P (1991). Toxicokinetics of permethrin in the rat. *Toxicol. Appl. Pharmacol.*, 31, 1-8.

Anadón A, Martinez-Larranaga MR, Diaz MJ, Bringas P, Fernandez MC, Martinez MA, Fernando-Cruz ML (1995). Effect of flumethrin on hepatic drug metabolizing enzymes and antipyrene disposition in rats. *Toxicol. App. Pharmacol.*, 132, 14-18.

Arafa HMM, Abd-Allah AR, El-Mahdy MA, Ramadan LA, Hamada FM (2003). Immunomodulatory effects of L-carnitine and q10 in mouse spleen exposed to low-frequency high-intensity magnetic field. *Toxicology*, 187, 171-181.

Aydogdu N, Atmaca G, Yalcin O, Taskiran R, Tastekin E, Kaymak K (2006). Protective effects of L-carnitine on myoglubinuric acute renal failure in rats. *Clin. Experim. Pharmacol. Physiol.*, 33, 119-124.

Bergmeyer HU (Ed.) *Methods of Enzymatic Analysis, 3rd Ed.*, Verlag-Chemie, Weinheim, pp. 154-160.

Boll M, Weber LWD, Messner B, Stampfl A (1998). Polychlorinated biphenyls affect the activities of gluconeogenic and lipogenic enzymes in rat liver: is there an interference with the regulatory hormone action? *Xenobiotica*, 28, 479-492.

Bucioli SA, De Abreu LC, Valenti VE, Vannucchi H (2012). Carnitine supplementation effects on nonenzymatic antioxidants in young rats submitted to exhaustive exercise stress. *J. Strength Condit. Res.,* 26, 1695-1700.

Cabaud PC, Wroblewski F (1958). Colorimetric measurement of lactate dehydrogenase activity of body fluids. *J. Clin. Pathol.*, 30, 234-236.

Cameron JS (1996). *Kidney Failure: The Facts*. Oxford University Press, New York.

Chhabra RS, Thompson M, Elwell NR, Gerken DK (1990). Toxicity of p-chloroamine in rats and mice. *Food Chem. Toxicol.*, 28, 717-722.

Czeczot H, Scibior D (2005). Role of L-carnitine in metabolism, nutrition and therapy. *Postepy Hig. Med. Dosw.*, 59, 9-19.

Deters M, Klabunde T, Kirchner G, Resch K, Kaever V (2002). Sirolimus/cyclosporine/tacrolimus interactions on bile flow and biliary excretion of immunosuppressants in a subchronic bile fistula rat model. *British J. Pharmacol.*, 136, 604-612.

Doumas BT, Watson WA, Biggs HG (1977). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinic. Chem. Acta.*, 31, 87-96.

El-Demerdash FM (2007). Lambda-cyhalothrin-induced changes in oxidative stress biomarkers in rabbit erythrocytes and alleviation effect of some antioxidants. *Toxicol. In vitro*, 21, 392-397.

El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH (2004). Role of α-tocopherol and β-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters and semen quality of male rats. *J. Environ. Sci. Health*, B39, 443-459.

Ellman GL (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, 82, 70-77.

Ellman GL, Courtney KD, Anders VJR, Featherstone RM (1961). A new rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, 7, 88-95.

Fahmy AM, Abdalla EF (2001). Cytogenetic effects by the natural pyrethrins and the synthetic lambda-cyhalothrin in mice in vivo. *Cytologia*, 66, 139–149.

Fossati P, Prencipe L (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.*, 28, 2077-2080

Giray B, Gurbay A, Hincal F (2001). Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E and allopurinol. *Toxicol. Lett.*, 118, 139-146.

Gupta RC, Goad JT, Kadel WL (1994). In vivo acute effects of carbofuran on protein, lipid, and lipoprotens in rat liver and serum. *J. Toxicol. Environ. Health*, 42, 451-62.

Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249, 7130-7139.

Haratym-Maj A (2002). Hematological alternations after pyrethroids poisoning in mice. *Ann. Agric. Environ. Med.*, 9, 199-206.

Hepler OE (1966). *Manual of Clinical Laboratory Methods*. C.C. Thomas Publishing, Springfield.

Henry RJ, Cannon DC, Winkelman JW (1974). *Clinical Chemistry. Principles and Technics, 11th ed*. Harper and Row Publishers, New York, 1629 pp.

Hossain MM, Suzuki T, Sato N, Sato I, Takewaki T, Suzuki K, Tachikawa E (2006). Differential effects of pyrethroid insecticides on extracellular dopamine in the striatum of freely moving rats. *Toxicol. Appl. Pharmacol.*, 217, 25-34.

Janardhan A, Rao AB, Sisodia P (1988). Short-term toxicity of methyl benzimidazole carbamate in dogs. *Bull. Environ. Contam. Toxicol.*, 41, 704-711.

Kalaiselvi T, Panneerselvam C (1998). Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. J. Nutr. Biochem., 9, 575–581.

Kale M, Rathore N, Jone S, Bhatnagar D (1999). Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxicol. Lett., 105, 197-205.

Keller JM, Kucklick JR, Stamper MA, Harms CA, McClella PD (2004). Association between organochlorine contaminant concentrations and clinical health parameters in loggerhead sea turtles from North Carolina, USA. Environ. Health Perspect., 112, 1074-1079.

Kidd H, James DR (Eds.) (1991). The Agrochemicals Handbook, 3rd Ed. Royal Society of Chemistry Information Services, Cambridge.

Knight JA, Anderson S, Rawle JM (1972). Chemical basis of the sulpha-phosphovanillin reaction estimating total serum lipids. *Clin. Chem.*, 18, 199-202.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 269-275.

Luskova V, Svoboda M, Kolarova J (2002). The effect of diazinon on blood plasma biochemistry in carp (*Cyprinus carpio* L.). *Acta Vet. Brno*, 71, 117-123.

Luty S, Latuszynska J, Halliop J, Tochman A, Obuchowska D, Przylepa E, Orczak E (1998). Toxicity of dermally applied alpha-cypermethrin in rats. *Ann. Agric. Environ. Med.*, 5, 109-115.

Maiti PK, Kar A (1997). Dual dose of testosterone in fenvalerate-treated mice with respect to thyroid function and lipid peroxidation. *J. Appl. Toxicol.*, 17, 127-131.

Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.*, 247, 3170-3175.

Moss DW (1984). In: Bergmeyer, H.U. (Ed.) *Methods of Enzymatic Analysis, 3rd Ed.*, Verlag-Chemie, Weinheim, pp. 92-106.

Nasuti C, Cantalamessa F, Falcioni G, Gabbianelli R (2003). Different effects of Type I and Type II pyrethroids on erythrocyte plasma membrane properties and enzymatic activity in rats. *Toxicology*, 191, 233-244.

Nice D (1997). Antioxidant based nutraceuticals. In: Yalpani, M., (Ed.). *New Technologies for Healthy Foods & Nutraceuticals*. Science Publishers, Shrewsbury, pp. 105-123.

Patton CJ, Crouch SR (1977). Spectrophotometeric and kinetics investigation of the Berthelot reaction for determination of ammonia. *Anal. Chem.*, 49, 464-469.

Pearlman FC, Lee RTY (1974). Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. *Clin. Chem.*, 20, 447-453.

Pournourmohammadi S, Nasser Ostad S, Azizi E, Ghahreman MH, Farzami B, Minaie B, Larijani B, Abdollahi M (2007). Induction of insulin resistance by malathion: Evidence for disrupted islets cells metabolism and mitochondrial dysfunction. *Pest. Biochem. Physiol.*, 88. 346-352.

Prasanthi K, Muralidhara, Rajini PS (2005). Fenvalerate-induced oxidative damage in rat tissues and its attenuation by dietary sesame oil. *Food Chem. Toxicol.*, 43, 299–306.

Principato GB, Asia MC, Talesa V, Rosi G, Giovannini E (1985). Characterization of the soluble alkaline phosphatase from hepatopancreas of *Squilla mantis* L. *Comp. Biochem. Physiol.*, B80, 801-804.

Raja M, Al-Fatah A, Ali M, Afzal M, Hassan RA, Menon M, Dhami MS (1992) Modification of liver and serum enzymes by paraquat treatment in rabbits. *Drug Metab. Drug Inter.*, 10, 279-291.

Rani PJ, Panneerselvam C (2002). Effect of l-carnitine on brain lipid peroxidation and antioxidant enzymes in old rats. *J. Gerontol A. Biol. Sci. Med. Sci.*, 57, 134-137.

Rebouche CJ, Seim H (1998) Carnitine metabolism and its regulation in microorganisms and mammals. *Annu. Rev. Nutr.*, 18, 39-61.

Rickard J, Brodie ME (1985). Correlation of blood and brain levels of the neurotoxic pyrethroid deltamethrin with the onset of symptoms in rats. *Pest. Biochem. Physiol.*, 23, 143-156.

Sadurska B, Boguszewski B (1993). Change in lipoprotein lipase activity and plasma liver lipids in thiram intoxicated rats. *Biochim. Pol.*, 40, 563-567.

SAS (1986) *SAS Users Guide: Statistics, version 5 Edition*. SAS Inst., Inc., Cary.

Satyanarayan S, Bejankiwar RS, Chaudhari PR, Kotangale JP, Satyanarayan A (2004) Impact of some chlorinated pesticides on the haematology of the fish *Cyprinus carpio* and *Puntius ticto*. *J. Environ Sci.*, 16, 631-634.

Sayim F, Yavasoglu NUK, Uyanikgil Y, Akyug H, Yavasoglu A, Turgut M (2005). Neurotoxic effects of cypermethrin in wistar rats: haematological, biochemical and histoppathological study. *J. Health Sci.*, 51, 300-307.

Seifert J (2001). Toxicologic significance of the hyperglycemia caused by organophosphorous insecticides. *Fundam. Appl. Toxicol.*, 7, 87-97.

Shakoori AR, Aziz F, Alam J, Ali SS (1990). Toxic effects of talastar, a new synthetic pyrethroid, on blood and liver of rabbit. *Pakistan J. Zool.*, 23, 289-300.

Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, Stevens JT, Weiner ML (2002). Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology*, 171, 3-59.

Sogorb AM, Vilanova E (2002). Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis, *Toxicol. Lett.*, 128, 215-228.

Steel RGD, Torrie JH (1981). *Principles and Procedures of Statistics. A Biometrical Approach, 2nd Ed*. McGraw-Hill Book Company, New York.

Symington SB, Frisbie RK, Lu KD, Clark JM (2007). Action of cysmethrin and deltamethrin on functional attributes of isolated presynaptic nerve terminals from rat brain. *Pestic. Biochem. Physiol.*, 87, 172-181.

Tappel AL, Zalkin H (1959). Inhibition of lipid peroxidation in mitochondria by vitamin E. *Arch. Biochem. Biophys.*, 80, 333-336.

Warnick GR, Benderson V, Albers N (1983). Selected Methods. *Clin. Chem.*, 10, 91-99.

Watson DA (1960). Simple method for the determination of serum cholesterol. *Clin. Chem. Acta*, 5, 589.

Wintrobe MM (1965). *Clinical Haematology*. Lea and Febiger, Philadelphia.

World Health Organization (1996). Fenvalerate. WHO/FAO Data Sheets on Pesticides, No. 95. International Program on Chemical Safety, Geneva, Switzerland.

Yousef MI, Awad TI, Mohamed EH (2006). Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. Toxicology, 227, 240-247.

Yousef MI, El-Demerdash FM, Kamel KI, Al-Salhen KS (2003). Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicology, 189, 223-234.