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MODULATION OF INTERLEUKIN-6 AND IMMUNOGLOBULINS IgG AND IgM LEVELS AFTER MULTIPLE ORAL EXPOSURE TO LAMBDA-CYHALOTHRIN IN RATS

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ABSTRACT

Lambda-Cyhalothrin (LCT) is a synthetic pyrethroid insecticide used worldwide in agriculture , home best , and disease vectors control . The objective of this study was to investigate the propensity of LCT to induce changes in some humoral immune parameters in adult male Sprague Dawley rats . Animals were divided into four groups (5 rats each) . The 1st and 3rd groups were orally given 1/10LD50 (12.76 mg/kg body wt.) every other day for 8 weeks. The 2nd and 4th groups were orally given dist. water every other day for 8 weeks and served as controls for the 1st and 3rd groups respectively . Blood samples were collected from each animal under ether anesthesia through heart puncture and sera were separated , at the 1st and the 15th days post LCT administration form groups 1 and 2 and groups 3 and 4 respectively . Data obtained revealed that oral LCT induced a significant decrease in IL-6, and significant increases in serum IgG and IgM concentrations. Significant increases in serum AST activity , total protein and albumin concentrations and non-significant decrease in serum ALT activity . Recovery from these changes were dependent on the measured parameter.

INTRODUCTION

Interleukin-6 (IL-6) is a poleiotropic cytokine with both pro and anti-inflammatory properties [(1)]. This cytokine is expressed by a variety of cells, including lymphocytes, monocytes, macrophages, neutrophils, endothelial cells, epithelial cells, and fibroblasts [(2)]. IL-6 is involved in modulating aspects of both innate and adaptive immunity via its ability to induce fever, B-cell differentiation and corresponding immunoglobulin production, T-cell activation and enhanced pro-inflammatory responses of neutrophils [(2,3)] .

The immunoglobulin's are proteins with anti-body activity; i.e they combine specifically with the substance that elicited their formation (immunogen or antigen), and they make up

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the humoral arm of the immune response. With the possible exception of "natural" antibody, antibodies arise in response to foreign substances introducedinto the body. They are therefore products of induced responses [(4)].

The two hallmarks of immunoglobulins are the specificity of each one particular antigenic structure and their diversity as a group, which meets the challenge of a vast array of antigenic structures in the environment. In addition to specifically binding antigens, the immunoglobulins express secondary biologic activities, which are important in defense against disease, e.g, transplacental passage, and facilitation of phagocytosis [(4)].

Lambda cyhalothrin, LCT, [(RS)-α-cyano-3-phenxybezyl-3-(2-chloro-3,3,3 – trifluoropropenyl)-2,2-dimethyl cyclopropane carboxylate] is a Type II pyrethroid insecticide . With regard to its effectiveness, it appears to be the first choice insecticide used than organochlorines , organophosphates and carbamates . It is widely used to control insect pests in agriculture , homes , gardens and disease vectors [(5)] .

The immune system has been shown to be an important target organ of toxicity following exposure to chemicals [(6,7)]. Although there is a large body of literature addressing immune responses during exposure to a wide variety of insecticides including carbamates [(8)], organochlorines [(9)], pyrethroides [(10)], organophosphorous compounds [(11)], and more recently neonicotinoids [(12,13,22)] · There have been few studies on the effects of LCT on the immune system. Its administration via food to male rabbits has been found to have adverse action on humoral and cell mediated immune responses as was measured by determination of the antibody titre against sheep red blood cells (sRBS) and delayed – type hypersensitivity reaction to tuberculin respectively [(14)]. The objective of this study is to further examine humoral immune response to multiple oral administration of LCT by monitoring its modulatory effects on serum IL-6 , IgG and IgM levels.

Material and Methods

Male Sprague – dawley rats weighing 150-200 g were used in this study. They were purchased from the animal house of the National Research Center, Dokki, Giza, Egypt. Rats were housed under standard laboratory conditions. They had free access to a standard food and provided with tap water *ad libitum*. All animals were acclimatized to these conditions for 10 days before being used.

Lambda cyhalothrin (LCT) was purchased from Shoura Co. , for chemicals, Kilo 28 Cairo – Alex desert Road, Egypt.

The acute oral LD_{50} of LCT for male Sprague – dawley rats was determined according to the method of Reed *et al.*,[(15)] and was found to be 12.76 mg/kg body wt.

Rats were divided into four groups (5 rats each) . The 1st and 3rd groups were orally given 1/10LD50 (12.76 mg/kg body wt.) every other day for 8 weeks. Animals of the 2nd and 4th groups were orally given dist. water every other day for 8 weeks and served as controls for Animals of the 1st and 3rd groups Five rats were picked up randomly from the 1st and 2nd groups at day one and from the 3rd group after 15 days (recovery period) following the termination of LCT administration . These animals were deprived of food for overnight and slightly anaesthetized with ether and blood samples were collected through heart puncture into plain vaccutainer tubes and centrifuged at 1500 x g for 15 minutes. Sera samples were separated in eppendorfs and kept at -80° c till the immunological and biochemical analysis were started.

Measurement of serum IL-6 concentrations of control and treated rats was carried out using AviBion Enzyme – Linked Immunosorbent Assay (ELISA) kits (Orgenium Laboratories, Helsinki, Finland), according to the manufacturer's instructions. Determination of serum immunoglobutins IgG and IgM concentrations of control and treated rats were carried out by immunonephrometry on the BN ProSpec System Following the method described by Dade Behring GmbH, Marburg, Germany. Following the method described by Thomas [(16)].

Serum ALT and AST activities and Serum total protein and albumin concentrations were measured by Dimension RxL Max/Max Suite Integrated Chemistry System manufactured by Dade Behring/2003/California, **U.S.A** , according to the methods described in kits manufacturer's instructions.

Data of each group were statistically analyzed using student "t" test according to Hill,[(17)] after one way analysis of variance (ANOVA) when multiple comparison was made according to Kirkwood[(18)]. Results were considered statistically significant at $P < 0.05$.

Results

Data presented in table 1 indicate that repeated oral administration every other day of LCT to male rats at the dose level of 1/10 LD_{50} (1.276 mg/kg b.wt.) for 8 weeks caused significant decreases in serum IL-6 and IgM, and a significant increase in serum IgG concentrations, at the first day post treatment termination with respect to the control value of

each parameter measured. However, no significant change was recorded in either of these parameters after 15 days (recovery period) post treatment termination .

Data presented in table 2 indicate that repeated oral administration every other day of LCT to male rats at the dose level of 1/10 LD_{50} (1.276 mg/kg b.wt.) for 8 weeks induced insignificant and significant increases in serum ALT and AST activities respectively . These effects lasted for 15 days (recovery period) post treatment termination .

Data presented in table 3 indicate that repeated oral administration every other day of LCT to male rats at the dose level of 1/10 LD_{50} for 8 weeks caused significant increases in serum total protein and albumin concentrations. These effects lasted for 15 days (recovery period) post treatment termination

Discussion

As a general rule , all the proposed approaches for immunotoxicity studies in human are based on the measurement of serum or plasma , cellular and functional immune parameters . Serum or plasma parameters include levels of cytokines , immunoglobulins , autoantibodies and inflammation markers [(19)] .

Data obtained in the present investigation indicate that repeated oral administration of LCT to male albino rats every other day for 8 weeks at a dose level of 1/10 LD induced a significant decrease in serum IL-6 when compared with its level in the control animals at the 1st day post-treatment period , but had no significant effect after 15 days (recovery period) post treatment termination.

Opposite to this observation , increased plasma or serum IL-6 level was previously recorded in male rats administered with carbon tetrachloride (CCl_4) either subcutaneously [(20)] , or orally [(21)] , or in rats administered orally with the neonicotinoid insecticide acetamiprid [(22)] .

Although the mechanism underlying the LCT – induced decrease of serum IL-6 remains unclear . It is well established that IL-6 plays an important role in several processes involved in hepatic physiology [(23)], including liver regeneration [(24)], acute phase responses [(25)] and hepatoprotection [(26; 27)]. The protective activity of IL-6 against carbon tetrachloride CCl_4 induced hepatotoxicity was supported by the findings that IL-6 knockout mice are subject to more intense liver injury and lipid peroxidation than wild type mice upon CCl_4 administration [(28 , 29)]. Moreover IL-6 administration before CCl_4 insult , inhibits CCl_4 – mediated apoptosis and accelerates hepatocyte regeneration in the liver of both IL-6 knockout and wild type mice [(29)] .

It has been previously reported that numerous cytokines are involved in pathologic processes induced by CCl_4 or regeneration of injured liver [23]. One such cytokine is IL-6, which is a major regulator of the acute – phase response [25] and has been considered to play a protective role in liver injury chemically induced by CCl_4 [28, 29, 30] or acetaminophen [31], as well as in liver injury immunologically induced by concanavalin A [3 , 32]

Hojo et al., [20] showed that subcutaneous CCl4 administration to rats induced IL-6 production and the level of IL-6 produced seemed to be inversely correlated with the hepatic marker enzymes level in plasma.

This endogenously induced IL-6 was suggested to suppress the development of liver injury[20]

Various cells such as monocytes, macrophages, fibroblasts, keratinocytes, endothelial cells, smooth muscle cells, T cells, B cells, granulocytes, mast cells and mesothelial cells can potentially produce IL-6 [1, 33.; 34] . A possible suppression of IL-6 release from these cells by LCT administration could explain the present decrease of this cytokine .

The present LCT toxicity was observed to induce a significant increase in serum IgG level concomitant with insignificant decrease in IgM level at the 1st day post-treatment period and lasted for 15 days (recovery period) post treatment termination .

This result disagrees with our recent observation that every other day oral administration of acetamiprid for six weeks induced a significant decrease in serum IgG level concomitant with insignificant increase in IgM level [22] , but agrees with that obtained by Mohany et al[13], who reported a significant increase in serum level IgG without any significant change in IgM in rats treated with imidacloprid, they attributed the increase in immunoglobulin to the formation of anti-imidacloprid antibodies. Elevation in serum IgG concentration, was also observed in workers exposed to multiple insecticides , These elevations have been attributed to inflammation induced by these insecticides [35] . Ig increase was previously attributed to lymphocyte activation in mice treated with propoxure [36],

The production of pro-inflammatory cytokines is a critical physiological process to orchestrate immune and metabolic responses during development, tissue regeneration, healing, trauma or infection, and to protect our bodies against hemorrhage, ischemia, cancer and sepsis. A controlled production of pro-inflammatory cytokines, such as interleukins (IL-

6) and tumor necrosis factor-alpha (TNF-a) trigger beneficial inflammatory responses that promote local coagulation to confine infection and tissue damage [(37)]

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Table 1 : Effects of repeated oral administration of lambda cyhalothrin (LCT) every other day for 8 weeks at the dose level of 1.276 mg/Kg body wt.) on serum IL-6 , IgG and IgM concentrations .

Parameter	Days Post-Treatment	Control	Treated	% Change
IL-6 (pg/ml)	1	**9.42±1.56**	**4.02±0.48** [a]	**- 57.32**
	15	**9.60 ±0.533**	**9.16±1.47** [a-b+]	**- 4.58**
IgG (g/l)	1	**2.12±0.192**	**2.8±0.122**[a]	**32.07**
	15	**2.08±0.256**	**1.96±0.288** [a–b+]	**- 5.769**
IgM (g/l)	1	**0.46±0.05**	**0.32±0.04**[a+]	**- 30.43**
	15	**0.50±0.126**	**0.48±8.36**[a-b+]	**- 4.00**

** Results are expressed as Mean ± SE of 5 samples*

a+ significant when compared with the control values ($p \leq 0.05$)

a- non significant when compared with the control values ($p > 0.05$)

b+ significant when compared with the LCT treatment values ($p \leq 0.05$)

Table 2 : Effects of repeated po administration of lambda cyhalothrin (LCT) every other day for 8 weeks at the dose level of 1.276 mg/Kg body wt.) on serum ALT and AST activities .

Parameter	Days Post-Treatment	Control	Treated	% Change
ALT (U/L)	1	61.1±2.34	58.6±4.53 $^{a-}$	- 4.09
	15	60.38 ±1.38	57.5±5.77 $^{a-b-}$	- 4.77
AST (U/L)	1	71.96±6.59	121.90±6.85^{a+}	69.40
	15	72.18 ± 5.73	94.48±6.91^{a+b+}	30.90

** Results are expressed as Mean ± SE of 5 samples*

a+ significant when compared with the control values (p ≤ 0.05)

a- non significant when compared with the control values (p > 0.05)

b+ significant when compared with the LCT treatment values (p ≤ 0.05)

b- non significant when compared with the LCT treatment values (p > 0.05)

Table 3 : Effects of repeated oral administration of lambda cyhalothrin (LCT) every other day for 8 weeks at the dose level of 1.276 mg/Kg body wt.) on serum total protein and albumin concentrations .

Parameter	Days Post-Treatment	Control	Treated	% Change
Total Protein (g/dl)	1	5.08±0.27	6.82±0.30^{a+}	34.78
	15	5.16±0. 34	6.84±0.27^{a+b-}	32.25
Albumin (g/dl)	1	2.08±0.21	3.28±0.23^{a+}	57.69
	15	2.14±0.24	3.22±0.15 $^{a+b-}$	50.47

** Results are expressed as Mean ± SE of 5 samples*

a+ significant when compared with the control values (p ≤ 0.05)

b- non significant when compared with the LCT treatment values (p > 0.05)