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Research Article

Immunotoxicity of Lambda-Cyhalothrin in Wistar Albino Rats

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ABSTRACT

Immunotoxicity of lambda-cyhalothrin was conducted in wistar albino rats @ dose level of 10 mg kg^{-1}. The parameter for humoral immunity such as haemagglutination test and total globulin estimation whereas for cellular immunity intradermal tuberculin test and in-vivo/in-vitro splenocytes proliferation test. The haemagglutination showed significant ($p<0.05$) inhibition of haemolysin titre and gamma globulin concentration. The cellular immunity exhibit significant ($p<0.05$) decrease in skin thickness in tuberculin test, total lymphocyte, % lymphocyte in treated group. Histopathological examination of adrenal showed congestion in modularly areas, infiltration of inflammatory cells and some necrotic area also observed in cortex and medullar region, focal concentration of the neurocytes' cytoplasm with pyknosis and disappearance of some purkinje cells. Proliferation of glial cells, infiltration of inflammatory cells in brain. Microscopic examination of kidney showed tubular haemorrhage, congestion in glomeruli, glomerular atrophy, infiltration of inflammatory cells and tubular degeneration. The microscopic examination of liver of rats revealed degeneration of hepatocytes, condensed nuclei, vacuolation and congestion in the central vein, spleen of rats exhibited atrophic changes in the white pulps characterized by decreased size of lymphoid follicles and periarteriolar lymphoid sheath (PALS), red pulp showed marked hemorrhages and hemosiderosis with a relatively increased collagenous connective tissue stroma between the narrow compressed vascular spaces were also seen and depletion of lymphoid follicles with small sized PALS. The cortical atrophy, characterized by necrosis of cortical lymphocytes with large clumps of nuclear debris to form "starry sky" appearance of cortex. Subsequent to thymicnecrosis, the cortex appeared thin as compared to control. Thymic lobules showed progressive atrophy. The tubular structure lined by epithelial cells was dilated and contained a homogenous eosinophilic material were observed in thymus gland.

INTRODUCTION

Agrochemicals and environmental chemicals affect immune responses have aroused considerable interest. Indirect immunological alterations which may be associated with low level pesticide exposure have not been completely evaluated and might cause break down to vaccination[1] and increased susceptibility to infection[2]. Therefore, immunosuppression by pesticides and other chemicals of environmental importance is a developing concern in toxicity assessment[3, 4]. Cyhalothrin is a type II pyrethroid used predominantly on cattle and sheep and to a lesser extent on pigs and goats for the control of a broad range of ectoparasites, including flies, lice, and ticks[5].

Lambda-cyhalothrin has broad spectrum insecticidal activity as cyhalothrin, but it is more active[6]. The acute oral toxicity of lambda-cyhalothrin is higher than that of cyhalothrin. Clinical signs of cyhalothrin and lambda-cyhalothrin toxicity include ataxia, unsteady gait, hyperexcitability, piloerection, subdued behaviour, salivation, Previous findings indicated that most type II pyrethroids such as cypermethrin, supercypermethrin forte, deltamethrin and fenvalerate, display immunosuppressive effects on humoral and cell mediated immune response in adult mice, rats, rabbits and goats[7, 8, 9, 10]. Some pyrethroids have also been reported to cause lymph node and splenic damage as well as carcinogenesis and mutagenesis[11].

There is no available information on the immunotoxicological effects of lambda-cyhalothrin, although acute, sub-acute and other toxicological information are available. The pyrethroids are neurotoxic and a close relationship between the nervous system and the immune system exists[12, 13]. Therefore, we have investigated the effects of the type II pyrethroid insecticide lambda-cyhalothrin on humoral and cellular immune responses.

MATERIALS AND METHODS

Humoral Immune Response

Haemagglutination test: Twelve healthy wistar albino rats of body weight 200 ± 20 were selected for study and divided into two groups. Group-I treated with vehicle control whereas group-II treated with lambda-cyhalothrin orally @ 10 mg/kg b.wt for period of 56 days. Both control and treated animals were immunized after 4 weeks of experiment by intraperitoneal injection with 0.2 ml of a 20% SRBC suspension in Alsever's solution. Ten days

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Table 1: Effect of lambda-cyhalothrin (10 mg/kg) on SRBC humoral immune response in rat by Haemagglutination test

Groups/Treatment	Antibody Titre
Vehicle control	256.00 ± 8.00
Lambda-cyhalothrin (10 mg/kg)	74.67* ± 6.66

Figures in parentheses indicate different treatment dosages.
Data with similar superscripts in a column did not differ significantly (P>0.05).

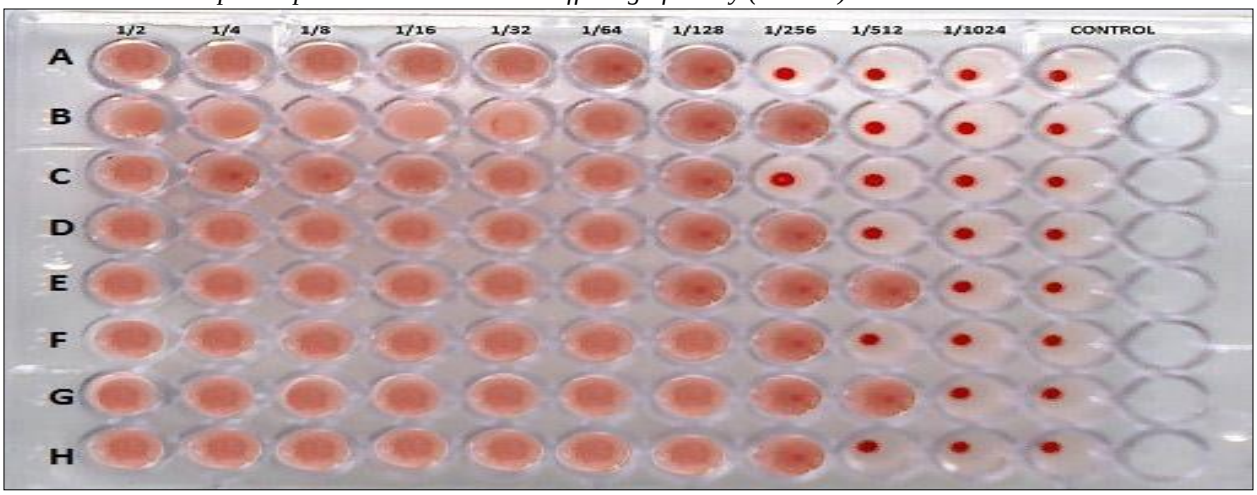

Photo 1: Showing haemagglutinin titre in vehicle control group against sheep RBC antigen in wistar albino rats

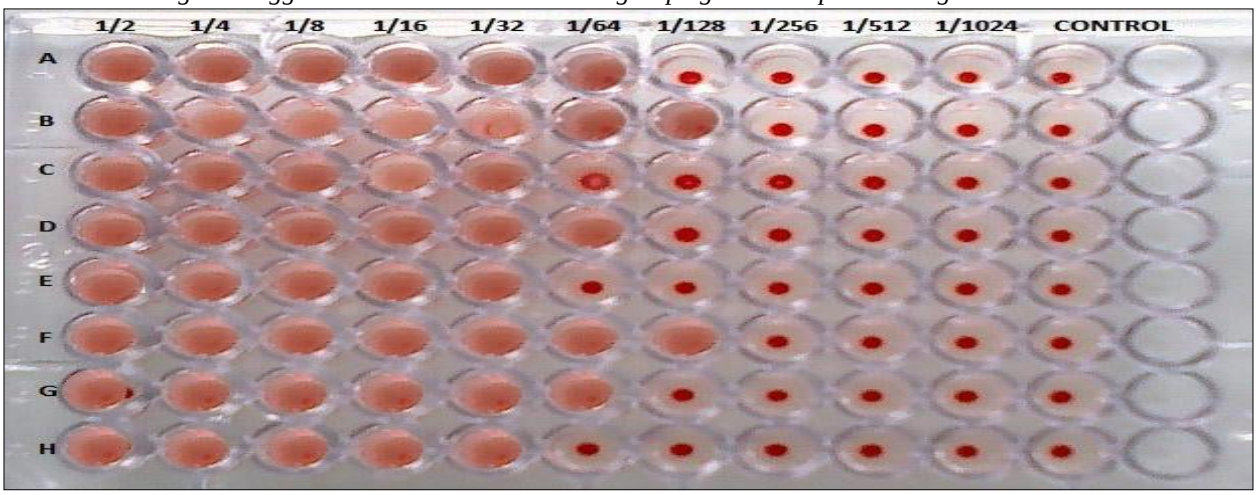

Photo 2: Showing haemagglutinin titre in lambda-cyhalothrin treated group against sheep RBC antigen in wistar albino rats.

after immunization, blood samples were collected from the orbital plexus and serum was removed to determine antibody titre by haemagglutination test (HA)[14].

Estimation of Gamma Globulin: Estimation of globulin was carried out by following the method[15]:

- The5.7 ml ammonium sulphate-sodium chloride solution [19.5% (NH4)2 SO_4 and 2.03% NaCl; pH 6.45] and 0.3ml clear serum was taken in a test tube. The contents were mixed gently by inverting the tube six times and kept on ice bath for 15 minutes. The tube was centrifuged at 3000 rpm for 10 minutes in a clinical centrifuge and supernatant was discarded. The same process was repeated twice and finally sediment was dissolved in 2 ml normal saline solution.
- Five ml Biuret reagent was added and kept for 10 minutes at room temperature.
- A blank was made with 2 ml normal saline and 5 ml Biuret reagent in tube, marked as 'B'.
- A standard was prepared by taking 2 ml of 0.15% bovine serum albumin and 5 ml of Biuret reagent in test tube and marked as 'S'.
- The absorbance of test and standard against blank set at 'zero' at 555 nm wavelength was taken.

$$\text{Serum gamma globulin (Gram/100 ml)} = \frac{\text{OD of test}}{\text{OD of standard}}$$

Cell-Mediated Immune Response **(CMI):**

Intradermal tuberculin test: Cell mediated immune response was evaluated in same animals in which humoral immune response was conducted[16]. Group-I treated with vehicle control whereas group-II treated with lambda-cyhalothrin orally @ 10 mg/kg b.wt for period of 56 day. Tuberculin solution was injected intradermal at 0.1 ml in the clipped flank on days 42 and 56 of experiment. The diameter and thickness of skin reactions were measured

Table 2: Effect of lambda-cyhalothrin (10 mg/kg) on gamma globulin concentration in rat

Groups/Treatment	Gamma globulin
Vehicle control	3.80a ± 5.13
Lambda-cyhalothrin (10 mg/kg)	1.98b ± 6.66

Figures in parentheses indicate different treatment dosages
Data with similar superscripts in a column did not differ significantly (P>0.05)

Table 3: Effect of oral administration of lambda-cyhalothrin (10 mg/kg) on tuberculin induced delayed type hypersensitivity response in rats

Groups/Treatment	Skin thickness (mm)
Group-I Vehicle control	5.485±0.49
Group-II Lambda-cyhalothrin (10 mg/kg)	3.182±0.55*

Figures in parentheses indicate different treatment dosage.
Data presented are mean ± SE of six animals in each group.
Data with small superscripts in a column differ significantly (P<0.05).*

Table 4: Effect of oral administration of lambda-cyhalothrin (10 mg/kg) for 56 days on total lymphocytes count, T and B lymphocytes count.

Lymphocytes Enumeration	Groups/Treatment (Mean ± SE)	
	Group-I Vehicle Control	Group-III Lambda-cyhalothrin (10 mg/kg)
Total lymphocytes	77.3±2.87	54.5±3.19*
T lymphocytes	44.8±1.68	27.7±2.01*
% of T lymphocytes count	58.1±1.85	50.6±1.45*
% change in T lymphocytes count	-	- 12.9
B lymphocytes count	14.5±0.89	7.50±0.5*
% of B lymphocytes count	18.9±1.12	13.8±0.59*
% change in B lymphocytes count	-	- 26.9

Figures in parentheses indicate different treatment dosages
Data presented are mean ± SE of five animals in each group.
Data with different superscripts in a row differed significantly (P<0.05).

with a caliper 24 hours after tuberculin injection.

Further, blood sample from each animal were collected from orbital plexus for enumeration of total lymphocyte, B and T lymphocyte. All animal were sacrificed on day 56 to perform gross pathological changes and histopathological changes of liver, thymus, kidney, spleen, adrenal and brain.

Enumeration of total lymphocytes and separation of T and B lymphocytes: Blood samples from rats of both groups were collected from retro-orbital plexus puncture in sterile test tubes containing EDTA @ 1-2 mg/ml of blood and immediately processed for total lymphocytes count and T and B lymphocytes separation.

Procedure for lymphocytes separation and counting: Lymphocytes were separated by using the commercially available Nylon wool fiber column (Polyscience Inc, Nulife, USA) employing the experimental protocol as described below:

Three ml of whole blood was properly mixed with five ml of PBS and layered on to 3ml of histopaque 1077 in a 15 ml conical centrifuge tube. It was centrifuged at 400 g (1550 rpm) for 30 minutes at room temperature. Supernatant was aspirated and discarded with the help of pasture pipette. The opaque zone was collected and transferred to a clean glass centrifuge tube and resuspended in 10 ml PBS. The tube was again centrifuged at 250g (800 rpm) for 10 minutes at 4ºC and again the supernatant was discarded. This procedure was repeated twice. Finally, the pellet was suspended in 2 ml RPMI-1640 medium and total lymphocytes were counted using haemocytometer.

Procedure for T and B lymphocytes separation: Syringe and plunger was taken out from sterile pack. The stopcock from the sterile package was removed and placed on the luer tip of the syringe. Column was washed with RPMI-1640 medium with 10% foetal calf serum at 37ºC. It was gently tapped while washing the column to ensure that the wool was wet and free from air bubbles. Stopcock was closed and the prepared column was incubated for one hour at 37ºC. Stopcock was opened to drain the media fully and then closed. Two ml viable lymphocytes suspension was added and the stopcock was opened to allow the media to drain until the cell volume had entered the packed wool. Again the stopcock was closed and top of the column was washed with an additional one ml of media and the wash was allowed to enter the packed wool. The stopcock was closed and another 2-5 ml of media was added to the column to ensure that the top of the wool was covered with media. Again it was incubated at 37ºC for one hour.

T lymphocytes which were non-adherent were collected by using two washings with RPMI-1640 medium (no plunging). Following collection of non-adherent T lymphocytes, 5-6 ml medium was added to fill the column and the column was knocked to dislodge the cells. Column was plunged and the procedure was repeated twice or thrice. T and B cells aliquots were then centrifuged separately at 1200 rpm for 10 minutes and the supernatant was discarded to collect the T and B cell pellets,

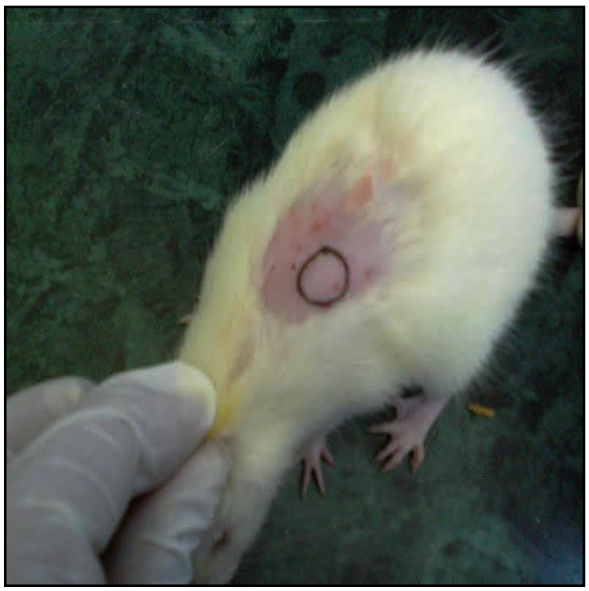

Photo 3: Intradermal inoculation of tuberculin in wistar albino rats on day 42nd in control group

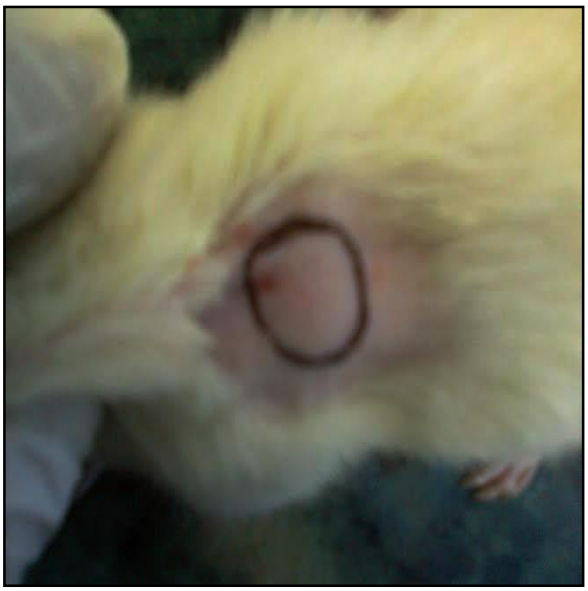

Photo 4: Intradermal inoculation of tuberculin in wistar albino rats on day 56th of in control group.

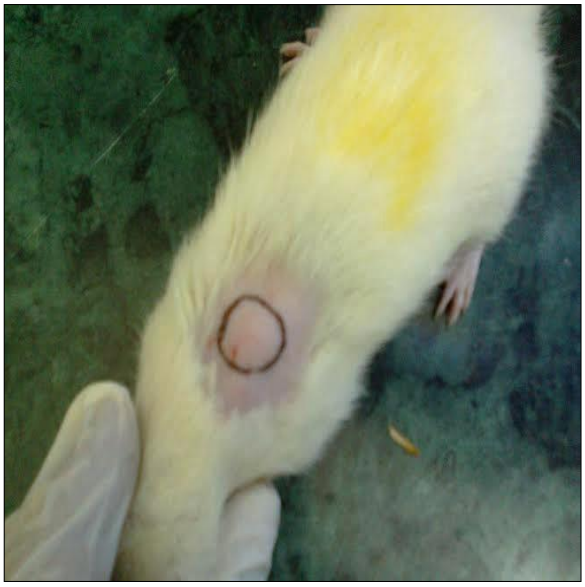

Photo 5: Intradermal inoculation of tuberculin in wistar albino rats on day 42nd in lambda-cyhalothrin treated group

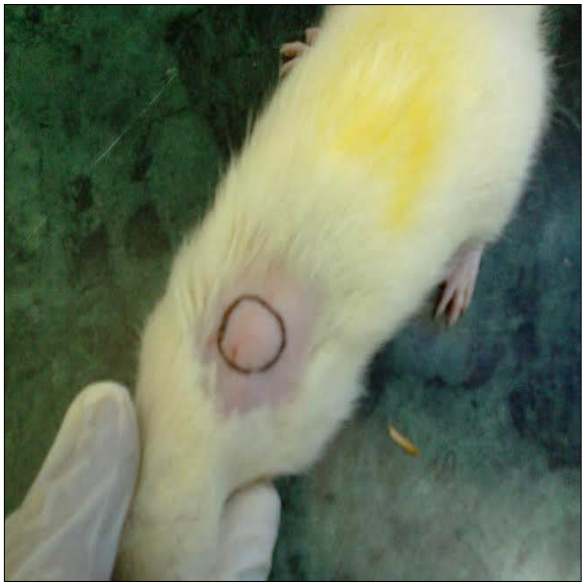

Photo 6: Intradermal inoculation of tuberculin in wistar albino rats on day 56th in lambda-cyhalothrin treatment group

respectively. T and B cells pellets were resuspended in 2 ml of RPMI-1640 medium and counted using haemocytometer.

RESULTS

Haemagglutination test: The haemolysin titre observed in test group was significantly ($p<0.05$) lower as compared to RBC control group Table 1 & Photo -1&2.

Estimation of Gamma Globulin: Results revealed that lambda-cyhalothrin (10 mg/kg) significantly ($p<0.05$) reduced gamma globulin concentration as compared to control (Table 2).

Cell Mediated Immune Response

Intradermal tuberculin test: the skin thickness observed in rats of control and lambda-cyhalothrin treated groups showed

significant ($p<0.05$) difference (Table-3 Photo 3-6).

Enumeration of total lymphocytes, T and B lymphocytes in rat: Effect of lambda-cyhalothrin on total lymphocytes, T and B cells counts are presented in Table 4

Total lymphocyte count: the total lymphocyte in control group I (77.3 ± 2.87), there was significant ($p<0.05$) decline in group II lambda-cyhalothrin (54.5 ± 3.19).

T lymphocyte count: Count of T lymphocytes in group I (44.8±1.68) was significantly ($p<0.05$) higher as comparative to group-II (27.7±2.01).

Percent T lymphocyte count: Compared to control group I (58.1±1.85), percent T lymphocytes count was significant ($p<0.05$) reduced in group II (50.6±1.45) by12.9%.

B lymphocytes count: The values of B lymphocytes observed in rats of group I (14.5±0.89) was significantly ($p<0.05$) higher as in group II (7.5 ±0.5).

Table 5: Effect of oral administration of lambda-cyhalothrin (10 mg/kg) for 56 days on Ex-vivo splenocytes proliferation in rat

Parameters	Control	Group/treatment	
		Cyclophosphamide (50 mg/kg)	Lambda-cyhalothrin (10 mg/kg)
OD	$0.21^{a} \pm 0.013^{a}$	0.145 ± 0.009^{b}	0.117 ± 0.007^{b}
Stimulation index	-	0.713 ± 0.062^{a}	0.573 ± 0.037^{b}

Figures in parentheses indicate different treatment dosages
Data presented are mean ± SE of eight animals in each group
Data with similar superscripts in a row did not differ significantly (P<0.05)

Percent B lymphocyte count: Compared to control group-I (18.9 ± 1.12), per cent B lymphocytes count in groups II (13.8 ± 1.15) were significant ($p<0.05$) lower by 26.9% as compared to control group.

Ex- vivo and in- vitro effects of lambda-cyhalothrin on splenocytes proliferation in rat

i) Ex -vivo effects: Ex vivo effect of lambda-cyhalothrin on splenocytes proliferation was evaluated and the mean values of OD and stimulation index are presented in Table-5 and Photo 7 &8. The mean OD values in Cyclophosphamide and lambda-cyhalothrin (50 and 20) treated groups II and III were 0.145±0.009 and 0.117±0.007 significant ($p<0.05$) lower as compared to the control group (0.21±0.013. The stimulation index values were found significant ($p<0.05$) differ in groups II (0.713±0.062) and group III (0.573±0.037), respectively.

ii) In -vitro effects: The mean OD values of in vitro splenocytes proliferation assay using four different concentration of lambda-cyhalothrin (5, 20, 100, 500 µg/ml) and stimulation index data are presented in Table-6 and Photo 9 & 10. Results revealed that there was significant ($p<0.05$) change in splenocytes proliferation at different concentrations of lambda-cyhalothrin as compared to control group. However, compared to control group-I. Lambda-cyhalothrin exhibited concentration dependent decrease in stimulation index values.

Histopathological Studies

Adrenal: Microscopic examination of adrenal showed congestion in modularly areas, infiltration of inflammatory cells and some necrotic area also observed in both cortex and medullar region (Photo 10 & 11).

Brain: Microscopic observation showed focal concentration of the neurocytes' cytoplasm with pyknosis and disappearance of some Purkinje cells. Proliferation of glial cells, infiltration of inflammatory cells and some haemorrhagic area were also observed (Photo 12 & 13).

Kidney: Microscopic examination showed tubular haemorrhage, congestion in glomeruli; glomerular atrophy, infiltration of inflammatory cells and tubular degeneration were observed (Photo 14 & 15).

Liver:Microscopic examination revealed degeneration of hepatocytes, condensed nuclei, vacuolation and congestion in the central vein. There were hemorrhages in sinusoidal spaces, condensation of nucleus, karyolysis, vacuolation and regeneration of hepatocytes was present in all animals of group III. Further, no microscopic changes were observed in vehicle control group (Photo 16, 17, 18 & 19).

Spleen: Microscopic examination exhibited atrophic changes in the white pulps characterized by decreased size of lymphoid follicles and periarteriolar lymphoid sheath (PALS) in comparison with the control group.

Red pulp showed marked hemorrhages and hemosiderosis with a relatively increased collagenous connective tissue stroma between the narrow compressed vascular spaces were also seen and depletion of lymphoid follicles with small sized PALS (Photo 20 & 21).

Thymus gland: Cortical atrophy, characterized by necrosis of cortical lymphocytes with large clumps of nuclear debris to form "starry sky" appearance of cortex. Subsequent to thymicnecrosis, the cortex appeared thin as compared to control.

Thymic lobules showed progressive atrophy. The tubular structure lined by epithelial cells was dilated and contained a homogenous eosinophilic material. (Photo 22 & 23).

DISCUSSION

Humoral immune response: The haemagglutination test used for the measuring of serum hemolysin titres against sheep red blood cells (SRBC). Sheep red blood cells (SRBC) are T-cell dependent antigen which needs cooperation of T-helper cells, B-cells and macrophages[17, 18]. The results indicated that lambda-cyhalothrin suppress haemolysin titre against SRBC as compared to negative control. The recorded reduction of humoral immune response confirmed the immunosuppression occurred after exposure to different type II pyrethroids in rats[7, 19, 20].

The reduction of gamma globulin in blood plasma can be confirm by the adverse cytotoxic effect of the lambda-cyhalothrin on the immunocomptent cells; more definitely the B-lymphocytes and plasma cells engaged in the production of various kinds of Immunoglobulins as suggested[20]. This assumption was ascertained by the previously established lowering effect of the insecticide on the haemolysin antibody titters in sera of treated rats as well as the lymphocytic depletion recorded in spleen of those rats.

The suppression of humoral immunity by lambda-cyhalothrin exposure was continual by the observed depletion of lymphoid cells in the white pulps of the spleen. The reduced humoral immunity can be attributed to inhibition of antibody production by plasma cells and / or inhibition of differentiation of B- Lymphocytes to plasma cells[21]. In addition, T-helper cells may be affected as SRBC is a T-cell dependent antigen[22].

Similar results in which marked thymocyte depletion were previously obtained in rats exposed to cypermethrin[7]. Moreover, high doses of cypermethrin, supercypermethrin forte and deltamethrin, displayed an immunosuppressive

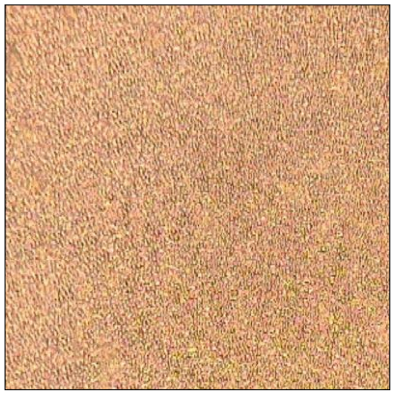

Photo 7: Ex-vivo splenocytes proliferation assay showing stimulated splenocytes in control group

Photo 8: Ex-vivo splenocytes proliferation assay showing decrease in splenocytes density and formazone crystals formed within the splenocytes in lambda-cyhalothrin treated group

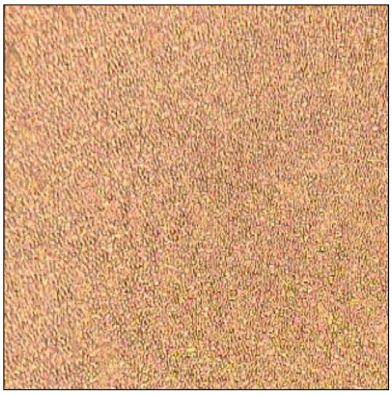

Photo 9: In-vitro splenocytes proliferation assay showing stimulated splenocytes in control group

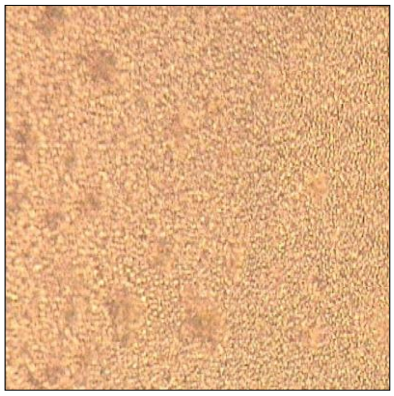

Photo 10: In-vitro splenocytes proliferation assay showing decreased stimulated splenocytes in lambda-cyhalothrin treated group

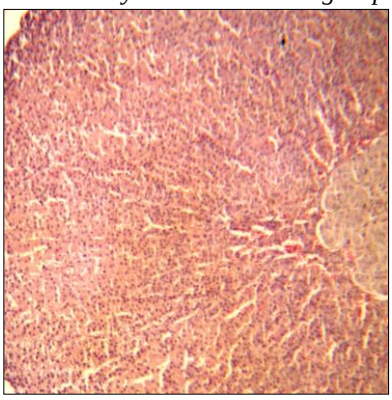

Photo 11: T.S. of adrenal showing normal modularly and cortical cell, no microscopic change was observed in control group of rats. 100X H&E

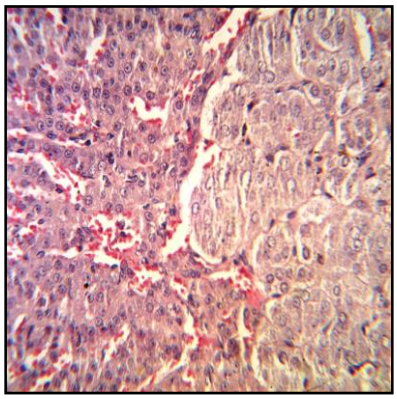

Photo 12: T.S. of adrenal showing congestion in modularly and cortical cell in treated group of rats. 1000X H&E

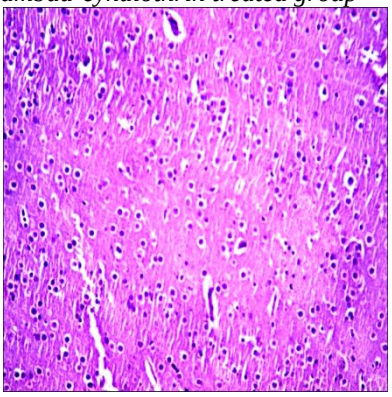

Photo 13: T.S of brain showing normal neuronal structure in control group of wistar albino rats H&E 100X

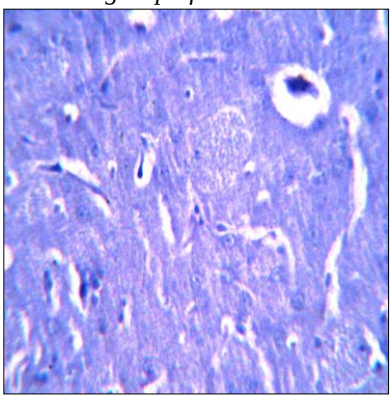

Photo 14: T.S of brain showed focal concentration of the neurocytes' cytoplasm with pyknosis and disappearance of Purkinje cells, proliferation of glial cells, hemorrhagic in wistar albino rats H&E 100X

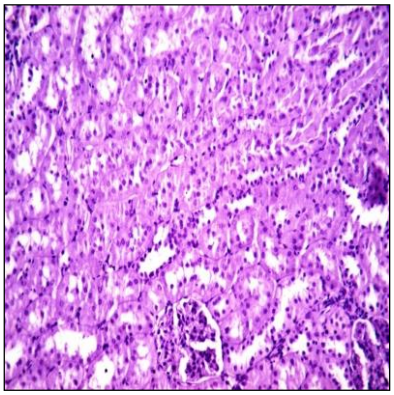

Photo 15: T.S of kidney showing normal glomerular structure, tubular and cellular architecture control group. H&H (100X)

effect on cell-mediated immune response in adult mice, rats and goats[1, 7, 8, 19]. Also, marked lymphocyte depletion was observed in the thymus and lymph nodes of cypermethrin–treated rats[7, 8]. A significant decrease in the total leukocyte count was also reported after cypermethrin exposure of rats[1].

Cellular immune response: The cell mediated immune was evaluated by tuberculin test, total lymphocyte, enumeration of B & T lymphocyte and splenocytes proliferation assay.

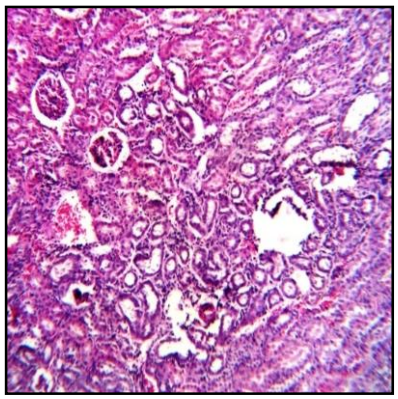

Photo 16: T.S. of kidney showing glomerular congestion, tubular haemorrhage and necrotic change in tubular cells in Lambdacyhalothrin treated group (100X)

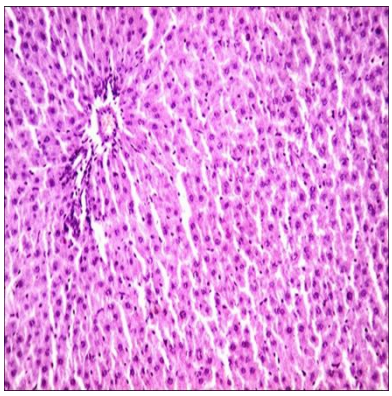

Photo 17: T.S of liver showing normal hepatocytes and hepatic cord in control group of wistar albino rats H&E 100X

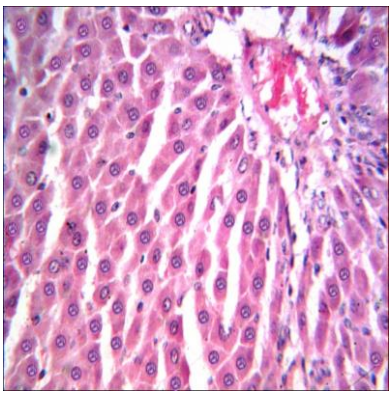

Photo 18: T.S of liver showing normal hepatocytes and hepatic cord in control group of wistar albino rats H&E 400X

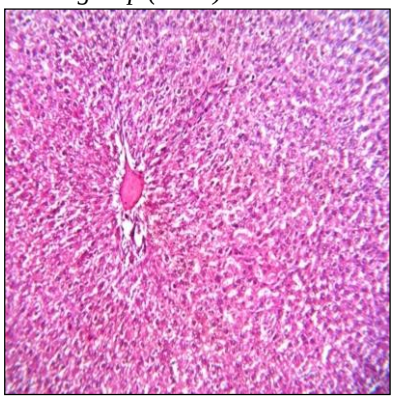

Photo 19: T.S. of liver showing degeneration of hepatocytes, condensed nuclei, vacuolation and congestion in the central vein in lambda-cyhalothrin treated group (100X)

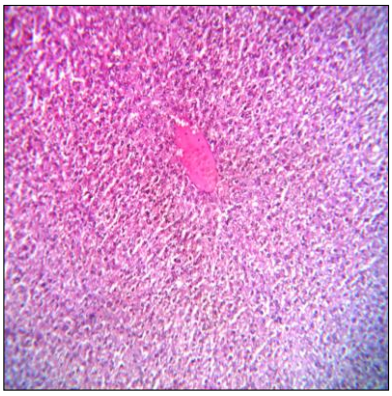

Photo 20: T.S. of liver showing degeneration of hepatocytes, condensed nuclei, vacuolation, hemorrhages in sinusoidal spaces, karyolysis and congestion in the central vein in lambda-cyhalothrin treated group. H&E (100X)

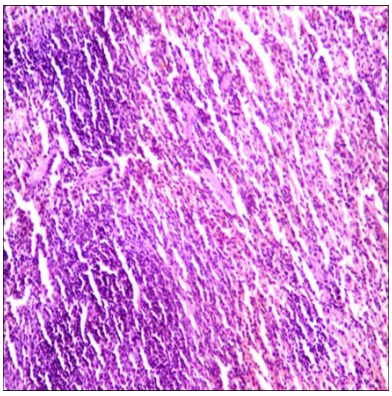

Photo 21: T.S. of spleen showing normal lymphoid tissue as well as cortical tissue in wistar albino rats treated with lambda-cyhalothrin at the dose level of 10 mg/kg b.wt H & E

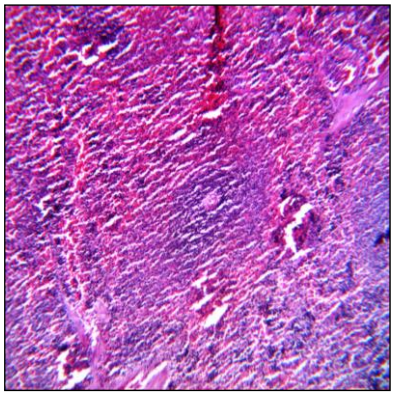

Photo 22: T.S. spleen showing atrophic changes in the white pulps characterized by decreased size of lymphoid follicles, red pulp showing marked haemorrhage and hemosiderosis with increase of collagenous connective tissue in lambda-cyhalothrin treated group. 100X H&E

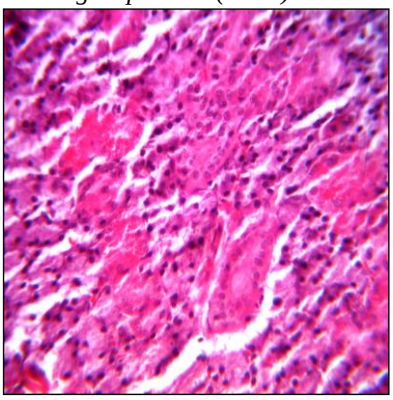

Photo 23: T.S. of thymus showing normal cortical structure and tubular structure in control group of wistar albino rats. H&E 400X

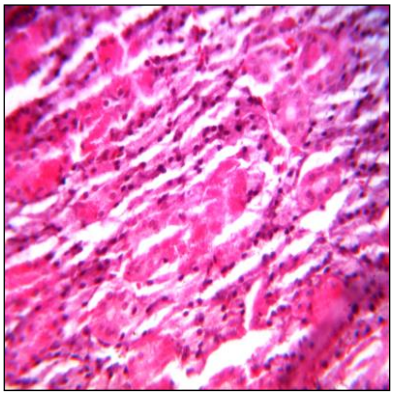

Photo 24: T.S. of thymus showing necrosis of cortical lymphocyte and tubular epithelial cells, eosinophilic homogenous material in tubules in lambda-cyhalothrin treated group of wistar albino rats. H&E 400X

Table 6: Effect of lambda-cyhalothrin (10 mg/kg) on in vitro splenocytes proliferation

Parameters	Control	Different concentration of lambda-cyhalothrin (µg)			
		5	20	100	500
OD	0.216±0.017[a]	0.182±0.002[b]	0.173±0.004[cb]	0.147±0.009[dbc]	0.118±0.003[e]
Stimulation index	-	0.848±0.067	0.811±0.081	0.691±0.092	0.582±0.025

Figures in parentheses indicate different treatment dosages.
Data presented are mean ± SE of five animals in each group.
Data with similar superscripts in a row did not differ significantly (P>0.05)

Lambda-cyhalothrin significantly inhibited tuberculin immune response in rats which is in close agreement with the results[23]. The suppression of cell-mediated immune response means inhibition of another subpopulation of T lymphocyte, the so - called T- effector cells[22] and/or inhibition of lymphokines production by activated T-cells[3]. The inhibited cell mediated immune response is supported by the recorded lymphopenia and histopathological changes in spleen. The depletion of lymphoid cells in thymus tissues as sensitized lymphocytes originate in the thymus and are formed active centres of spleen and lymph nodes[24].

In present investigation, lambda-cyhalothrin significantly depleted splenic T and B lymphocytes. The pesticide has ability to interfere with the release and/or production of some cytokines[19] and other factors which may be involved in alterations of splenic T and B cell distribution and proliferation.

It has been explained that most type II pyrethroids exert a direct effect on leucocytes, through an action on Na+ membrane channels and/ or by an indirect action on macrophage (key elements in cellular immune responses) activity via hypothalamic pituitary adrenals axis activation[25]. The suppressed cellular immune response observed in our study could be attributed to these effects. It has been reported also that the cytotoxic effects are based on the specific genotoxicity of pyrethroids which causes the immunosuppression[26, 27] confirmed the genotoxicity of lambda-cyhalothrin on human lymphocytes cultured in vitro[28]. Synergistic effects may aggravate immunotoxicity and allegro toxicity[29]. The xenobiotic capable of inducing peripheral neurotoxicity could potentially be capable of indirectly inducing immune alterations, by affecting the signals from nerve terminals to lymphocytes[30]. The interactions of pyrethroids with neuronal receptors may be an important regulator of immunomodulation[31]. This was sustained by the recorded brain lesions in our results.

Several lines of evidence indicate that type II pyrethroids are strong inducers of both adrenaline (A) and noradrenaline (NA) release[32, 33]; neurotransmitter release seems to be secondary to the increased Na+ entry[34], and depression of the resting chloride conductance, which amplifies sodium effects[35]. Noradrenaline is also synthesized and stored in the splenic and thymic nerve terminals[36].

In addition, alpha and beta-adrenergic receptors have been demonstrated on several immune cell type, including different subpopulations of T lymphocytes, granulocytes, monocytes, macrophages, and NK cells[37]. Output of neuroendocrine pathways has a modulatory effect on the migratory behaviour of lymphocyte in vivo. Thus, it can lead to rapid changes in the specific phenotype of lymphocytes accumulating in tissues and organs undergoing immune challenge[38].

Therefore, the reduced proliferative response observed in the spleen and thymus from lambda cyhalothrin exposed rat, could be the result of pyrethroid-induced catecholamine release. These catecholamine-mediated suppressive effects have been partially attributed to NA induced apoptosis[39].

The inhibitory effect of cypermethrin on the activity of the total ATP-ase in rat liver, which may disturb the active transport of Na^+, K^+ and Mg^+ ions and result in pathological changes in liver cells[40]. The recorded tissue damage in the liver and brain could be attributed also to the effect of reactive oxygen species (ROS) generated during this pyrethroid insecticide metabolism[41].

It has been reported that pyrethroids induce hepatotoxic effects with a consequent suppressive effect on plasma protein production and/ or albumin globulin ratio[42]. The pathological changes in liver may affect serum total protein and/ or albumin globulin ratio and consequently considered as one of the contributing causes of immunosuppression in our study.

In present study, microscopic changes were observed in liver, spleen, kidney, thymus and brain are in closed agreement with the results[10] found a dose-dependent increase in the liver weight after permethrin exposure of rats. Also, [43]confirmed the presence of parenchymatous degeneration with lymphoid infiltrations in the liver of rats and mice exposed to alpha-cypermethrin. Moreover, the brain of exposed rats showed focal concentration of the neurocytes' cytoplasm with pyknosis and disappearance of some Purkinje cells[44].

In conclusion, the presence of immune alterations subsequent to lambda cyhalothrin exposure at the tested doses suggests that sub-acute and/or chronic exposure to lambda cyhalothrin in the environment has the potential to alter immune function. Therefore, we advise to use this insecticide at the recommended field application levels away from vegetation to be eaten by animals and to minimize the direct exposure to it as much as possible in order to avoid its immunosuppressive effect.

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