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TOXICOKINETICS AND IMMUNOTOXICITY STUDY OF LAMBDA-CYHALOTHRIN IN WISTAR ALBINO RATS

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ABSTRACT

The toxicokinetics study of lambda-cyhalothrin and effect on immune response in lambda-cyhalothrin treated wistar albino rats was conducted at single oral dose 20 mg kg^{-1} and 10 mg kg^{-1}. The lambda-cyhalothrin was administered in overnight fasting rats by mixing in 0.1% Tween 80 and Serial blood samples were collected at 0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 24.0 hr after administration. Liver, kidney, heart, adrenal, spleen, brain, testis and ovaries samples were also collected at same time interval. The concentration of lambda-cyhalothrin concentrations were determined by HPLC. The humoral immune response and cellular immune was observed. The plasma and tissue concentration–time data for lambd-acyhalothrin were found to fit a one-compartment open model. The elimination half-life and mean residence time for lambda-cyhalothrin were found 5.09±0.16 h and 7.32±0.11 h, respectively. The mean volume of distribution (Vd_{area}) was calculated to be 3.94±0.12 L/kg. The total body clearance (Cl_B) was observed to be 0.54±0.01 $L.kg^{-1}.h^{-1}$. The maximum plasma concentration of lambda-cyhalothrin (Cmax) was noted to be 6.42±0.11 $\mu g.ml^{-1}$ at t_{max} of 2.00 ± 0.00 h exhibit that after oral administration, lambda-cyhalothrin was extensively but slowly absorbed. The maximum concentrations Cmax was observed highest in spleen (17.9±0.38), ovaries (9.77±0.81), liver (7.75±0.28), kidney (7.75±0.28), heart (4.90±0.42), adrenal (4.87±0.69), brain (6.63±0.25) and testis (1.46±0.10) $\mu g.ml^{-1}$. Tube agglutination test results showed significant ($p<0.05$) inhibition of antibody titre against *S. typhimurium* 'O' antigen. The cellular immune response *ex-vivo* and *in-vitro* effects of lambda-cyhalothrin on splenoc-

proliferation significant ($p<0.05$) decreased.

KEYWORDS: Lambda-cyhalothrin; toxicokinetics; body organs; tube agglutination test.

INTRODUCTION

Pyrethroids insecticide may be classified into two large groups [1, 2]. Type I pyrethroids (e.g. allethrin, permethrin) lack a cyano group. Type II pyrethroids (e.g. deltamethrin, fenvalerate and cyhalothrin) have a cyano group in the position. They are neurotoxic both for mammals and insects. Lambda-cyhalothrin [cyano-3-phenoxybenzyl 3-(2-chloro-3, 3, 3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] is a type II pyrethroid with a high level of activity against a wide range of Lepidoptera, Hemiptera, Diptera, and Coleoptera species. It has found extensive uses in public and animal health applications where it effectively controls a broad spectrum of insects and ectoparasites, including cockroaches, flies, lices, mosquitos, and ticks [3, 4].

Toxicokinetics (TK) of a chemical substance are conducted to obtain adequate information on its absorption, distribution, biotransformation (i.e. metabolism) and excretion, to aid in relating concentration or dose to the observed toxicity, and to aid in understanding its mechanism of toxicity. TK may help to understand the toxicology studies by demonstrating that the test animals are systemically exposed to the test substance and by revealing which are the circulating moieties (parent substance/metabolites). Basic TK parameters determined from these studies will also provide information on the potential for accumulation of the test substance in tissues and/or organs and the potential for induction of biotransformation as a result of exposure to the test substance. Adequate toxicokinetic data will be helpful to support the further acceptability and applicability of quantitative structure-activity relationships, read-across or grouping approaches in the safety evaluation of substances. Kinetics data may also be used to evaluate the toxicological relevance of other studies (e.g. *in-vivo/in-vitro*).

The ability of natural or synthetic agro-chemicals and other environmental chemicals to affect immune responses has aroused considerable interest. Subtle immunological alterations which may be associated with low level pesticide exposure have not been completely evaluated and might cause break down to vaccination [5] and increased susceptibility to infection [6]. Therefore, immunossupression by pesticides and other chemicals of environmental importance is a developing concern in toxicity assessment [7, 8, 9].

MATERIALS AND METHODS

Selection of Animals

The toxicokinetics study conducted in forty clinically healthy wistar albino rats (male & female) of body weight 200±10 gm were used. The animals were individually housed in polycarbonate cages with paddy bedding and were maintained in environmentally controlled rooms (22±2^{o}C and 50±10% relative humidity) with a 12 h light/dark cycle (light from 08:00 to 20:00 h). Food (Pranav) and online UV aqua guard water were made available *ad libitum*.

Drugs Used

Lambda-cyhalothrin [cyano-3-phenoxybenzyl 3-(2-chloro-3, 3, 3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] (99%) was provided by M/s Gharda Chemicals Limited, B-27/29, MIDC, Dombivli (E)-421 203 Distt. Thane, Maharashtra State (India) was used.

Experimental Design

Forty wistar albino rat selected after acclimatization divided into eight groups (Group-I, II, III, IV, V, VI, VII & VIII) of each five animals and lambda-cyhalothrin was administered separately in each of animals by oral route at the dose level of 20 mg/kg b.wt. The sample collections were made from respective group 0.25 h, 0.50 h, 1.0 h, 2.0 h, 4.0 h, 8.0 h, 16.0 h, 24.0 h respectively.

Administration of Drugs

Single dose of lambda-cyhalothrin @ 20 mg kg^{-1} in overnight fasted rats and water was allowed *ad libitum*. Lambda-cyhalothrin was administrated by gavage in a volume of 10 ml/kg b.wt in 0.1% Tween-80.

Collection of Biological Sample

Blood samples (approx.0.5 ml) were drawn from the orbital plexus into heparinized glass centrifuge tubes at 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16 and 24 h after administration of the lambda-cyhalothrin. Plasma was separated by centrifugation at 3,000 r.p.m. for 15 min at room temperature and kept at -4ºC until analysis, which was usually done on the day of collection**.**

Processing of Plasma

1. Carefully remove plasma and pipet 100 µl into a 1.5-ml micro centrifuge tube. Remaining plasma was frozen on dry ice and stored frozen for future use.
2. Add 200 µl chilled acetonitrile to plasma sample and vortex. Centrifuge the sample 10 min at 5000 rpm at 4 ºC.

3. Remove supernatant without disturb of pellet and place in appropriate auto injector vial for analysis.

Collection of Tissue Samples

All the animals of each group were killed by cervical dislocation immediately after blood collection at respective time interval. Different tissue samples (liver, kidney, spleen, adrenal, heart, brain, ovaries and testis) were collected at same time interval of blood collection.

Processing of Tissue Sample

1. Following removal, carefully weigh each tissue and place it into an appropriately sized container for homogenizing (1.5 to 50-ml centrifuge tubes).
2. Add *twice* volume of chilled PBS to the weight of the tissue (w/w) and homogenize using Polytron homogenizer.
3. Pipet 200 µl of homogenate into 1.5-ml micro centrifuge tubes and add 400 µl chilled acetonitrile to homogenized sample and vortex.
4. Centrifuge the sample 10 min at 5000 rpm.
5. Taking care not to disturb the pellet, remove supernatant and place in appropriate auto injector vial for analysis.

Estimation of Lambda-Cyhalothrin

HPLC-	Agilent Technology
Column-	Qualisil BDS C18, 4.6x250 mm, 5µm
Mobile phase-	Acetonitrile and water (80:20 v/v)
Flow rate-	1 ml/min
Detection-	Ultraviolet absorption at 235 nm

Plasma and tissue concentrations of lambda-cyhalothrin were measured by high-performance liquid chromatography described [10].

Calculation of Toxicokinetic Parameters

Toxicokinetic parameters of lambda-cyhalothrin after a single oral administration were calculated from semi log plot of plasma toxic concentration versus time curve. The experimental data were analyzed using a one compartment open model for lambda-cyhalothrin as described [10]. Following oral administration, the toxicokinetic parameters were calculated by one compartment open model by PK Solution Software Version 2.0.

Statistical Analysis

Comparison of concentrations of the drugs in plasma and tissue at different time intervals by using ONE WAY ANOVA followed by Tukey HSD test [11].

Immune Response: Humoral immune response

Tube Agglutination Test

1. Preparation of *Salmonella typhimurium* 'O' antigen was done as per method described [12]. The smooth colonies of *Salmonella typhimurium* grown on tryptose agar were washed with normal saline. Nutrient broth was inoculated with washed colonies and incubated at 37 ^{0}C. After 6-8 hours of incubation, tubes containing nutrient broth were boiled in water bath at 100 ^{0}C for two hours and thirty minutes. Killed *Samonella typhimurium* culture was then designated as 'O' antigen and used for determination of humoural immune response in rats.

2. Raising of antiserum against *Salmonella typhimurium* 'O' antigen:

Immunization of rats: Experimental rats were divided into two groups of six animals each. Rats of group 1 (negative control) received only distilled water and rats of group II was orally administered lambda-cyhalothrin 10 mg/kg for 21 days. Thereafter, rats of both these groups were immunized with *Salmonella* 'O' antigen along with continuance of lambda-cyhalothrin (10 mg/kg) treatment. The immunization schedule following is given below:

1st day: 1st dose of *S. typhimurium* 'O' antigen 1ml S/C at 3-4 sites.
7th day: 1st booster of *S. typhimurium* 'O' antigen 1ml S/C at 3-4 sites.
15th day: 2nd booster of *S. typhimurium* 'O' antigen 1ml S/C at 3-4 sites.
Blood samples were collected from all the rats by retro-orbital plexus puncture 15 days after the second booster and serum was separated for determining antibody titre using Tube agglutination test.

Tube Agglutination Test

1. Tube agglutination test was used for determination of serum antibodies titre.
2. Tubes were arranged in test tube stand in a row of 11 tubes each. With the help of micropipette, 0.8 ml normal saline was added to 1st tube and 0.5 ml in rest of tubes in a row.
3. 0.2 ml of rat antiserum (collected from animals of different groups) was added to 1st tube and mixed thoroughly.
4. Thereafter, 0.5ml of the mixture from first tube was transfer to second tube which and mixed thoroughly.

5. Similar process of transferred to the next tubes and mixing thoroughly was followed serially except the last 11th tube was kept as antigen control.
6. From the 10th tube, 0.5ml of mixture of saline and serum was discarded. After that, 0.5 ml of killed *Salmonella typhi* 'O' antigen was added to all the tubes and mixed thoroughly.
7. All the tubes were placed in a BOD incubator set at 37 ℃.
8. After 24-48 hours of incubation, agglutination titer was calculated.
9. The end titer was highest dilution showing at least 50 percent clearing. If agglutination did not occur and the antigen remained in suspension, it was considered as negative agglutination.
10. For reading the end titre, the last antigen control tube was compared with other tubes, and results were computed. Statistical analysis: The comparison between control and treated groups was made using the unpaired "t" test to measure the statistical significance of treatment effects [11].

***Ex Vivo* and *In-Vitro* Effects of Lambda-Cyhalothrin on Splenocytes Proliferation**

The study was conducted as per method described [13].

Effect of lambda-cyhalothrin on *Ex-vivo* studies: Adult rats of either sex weighing 100-120 gm were randomly divided into two groups of six animals each. Rats of group I (Control) were administered triple glass distilled water (TDW) while animals of group II were administered orally lambda-cyhalothrin 10 mg/kg b.wt, respectively for 56 days. After 42 days, animals of the all the three groups were sacrificed and spleen were aseptically collected and splenocytes were harvested as below.

i. Preparation of Splenocytes

1. Collect spleen tissues aseptically from rat and subsequently transfer to another sterile petridish containing medium.
2. Tease spleen tissues gently using a sterile needle and forceps to release cells from the tissues.
3. Allow it to stand for 2 min for regimenting clumps.
4. Then transfer the upper portion of the medium containing the splenocytes using 1 ml pipette, to a 15 mL sterile centrifuge tube.
5. Again, leave to stand for 2 min (in ice) and transfer to a fresh centrifuge tube.
6. Centrifuge the cell suspension at 1000 x g for 5 min, wash cells once again in basal culture medium and then 1ml of erythrocyte lysis buffer was added to the pellet.
7. After 30 to 45 sec, add 5 mL of culture medium to this and centrifuge at 1000 g for 5 min.
8. Wash the lymphocytes twice with culture medium to remove the traces of lysis buffer and

Finally suspend in 1 mL of RPMI 1640 containing 10% FCS.

9. Count cells by using a small aliquot of cell suspension.

ii. Dispensing And Culturing of Lymphocytes:

1. Make cell suspension containing $1x10^7$ viable cells/mL in culture media containing 10% FCS.
2. Dispense cells at the rate of 100 µL per well in 96 well flat bottomed tissue culture plates.
3. Allow cells to grow to determine the difference between cell proliferations.
4. Incubate plates in a humidified CO_2 incubator air atmosphere (5%) at 37°C for 48h.

iii. Cell Proliferation Assay

1. Add 10 µL of MTT solution to all wells of 48 h cultured lymphocytes and incubate for 4 h at 37°C.
2. During this period, formazon crystals will be formed at the bottom of each well.
3. Pipette out the spent media along with suspension of cultured cells.
4. Then add 100 µL of acid isopropanol (0.1 N HCl in anhydrous isopropanol) to all wells and mix thoroughly to dissolve the dark blue crystals.
5. After a few minutes at room temperature read plates using a plate analyzer in dual wavelength measuring system, at a test wavelength of 540 nm, a reference wavelength of 630 nm.
6. Read plates normally within 1 h of adding the acid isopropanol.
7. Calculate cell proliferation as stimulation index:

$$\text{Stimulation index} = \frac{\text{A540 nm with lambda-cyhalothrin}}{\text{A540 nm control}}$$

Where, A 540 = Absorbance at 540 nm.

Statistical analysis: The comparison between control and treated groups was made using the student's "t" test to measure the statistical significance of treatment effects [11].

Effect of Lambda-Cyhalothrin on *In-Vitro* Studies

Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is based on the ability of a

mitochondrial dehydrogenase enzyme (by generating reducing equivalents such as NADH and NADPH) from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue formazan crystals, which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. Solubilisation of the cells by the addition of a detergent results in the liberation of the crystals which are solubilized. The color can then be quantified using a simple colorimetric assay. The results can be read on a multiwell scanning spectrophotometer (ELISA reader). The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. Here, we took splenocytes for cell proliferation assay.

Reagents

i. Basal culture medium (RPMI-1640) pH 7.2 sterilized through 0.22 µm Millex-GV filter unit (Millipore). Store the prepared medium in small aliquots at 5˚C in dark.
ii. Erythrocyte lysis buffer, pH 7.2
iii. MTT Solution: MTT was dissolved in RPMI-1640 at 5 mg/mL and filter through 0.22 µm filters.
iv. Preparation of culture medium: Supplement the basal culture medium with 10% fetal calf serum (FCS-heat inactivated at 560 C for 30 min) for its use as culture medium and add mitogen i.e., lipopolysaccharide (LPS; 10-50 µg/mL) to proliferate the cells.

Preparation of Splenocytes

1. Collect spleen tissues aseptically from rat and subsequently transfer to another sterile petridish containing medium.
2. Tease spleen tissues gently using a sterile needle and forceps to release cells from the tissues.
3. Allow it to stand for 2 min for regimenting clumps.
4. Then transfer the upper portion of the medium containing the splenocytes using 1 ml pipette, to a 15 mL sterile centrifuge tube.
5. Again, leave to stand for 2 min (in ice) and transfer to a fresh centrifuge tube.
6. Centrifuge the cell suspension at 1000 x g for 5 min, wash cells once again in basal culture medium and then 1ml of erythrocyte lysis buffer was added to the pellet.
7. After 30 to 45 sec, add 5 mL of culture medium to this and centrifuge at 1000 g for 5 min.

8. Wash the lymphocytes twice with culture medium to remove the traces of lysis buffer and finally suspend in 1 mL of RPMI 1640 containing 10% FCS.
9. Count cells by using a small aliquot of cell suspension.

Dispensing and Culturing of Lymphocytes

1. Make cell suspension containing $1x10^7$ viable cells/mL in culture media containing 10% FCS.
2. Dispense cells at the rate of 100 µL per well in 96 well flat bottomed tissue culture plates.
3. Allow cells to grow with Negative control and lambda-cyhalothrin to determine the difference between cell proliferations.
4. Incubate plates in a humidified CO_2 incubator air atmosphere (5%) at 37°C for 48h.

Cell Proliferation Assay

1. Add 10 µL of MTT solution to all wells of 48 h cultured lymphocytes and incubate for 4 h at 37°C.
2. During this period, formazon crystals will be formed at the bottom of each well.
3. Pipette out the spent media along with suspension of cultured cells.
4. Then add 100 µL of acid isopropanol (0.1 N HCl in anhydrous isopropanol) to all wells and mix thoroughly to dissolve the dark blue crystals.
5. After a few minutes at room temperature read plates using a plate analyzer in dual wavelength measuring system, at a test wavelength of 540 nm, a reference wavelength of 630 nm.
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$$\text{Stimulation index} = \frac{\text{A540 nm with Lambda-cyhalothrin}}{\text{A540 nm control}}$$

Where, A 540 = Absorbance at 540 nm.

Statistical Analysis: Comparison of concentrations of the drugs in plasma and tissue at different time intervals by using ONE WAY ANOVA followed by Tukey HSD test [11].

RESULTS

Lambda-cyhalothrin various tissue concentrations versus time profile has confirmed one compartment model for lambda-cyhalothrin. The values of different toxicokinetics

parameters in albino rats calculated by the above noted compartment model. After oral administration of lambda-cyhalothrin, the mean extrapolated zero time concentration of lambda-cyhalothrin in serum during absorption phase (A') and elimination phase (B) was noted to be 5.48±1.51 and 5.96±0.46µg/ml, respectively. The absorption rate constant (Ka) were raised from 0.16 to 1.58 h^{-1} with a mean value of 0.57±0.21 h^{-1} while its elimination rate constant (β) ranged from 0.123 to 0.153 h^{-1} with a mean value of 0.14 ± 0.004 h^{-1}. The mean absorption half-life ($t_{½Ka}$) and elimination half-life ($t_{½\,\beta}$) was calculated to be 2.00±0.47 and 5.09±0.16 h, respectively.

The mean area under curve in serum (AUC) and mean area under first moment curve (AUMC) were noted to be 37.3±0.44 µg.ml^{-1}h and 272.8±6.31 µg.ml^{-1}.h^2, respectively with the mean residence time (MRT) of 7.32±0.11 h. The various values of volume of distribution calculated by different methods are shown in Table 1. The mean volume of distribution (Vd_{area}) was calculated to be 3.94±0.12 L/kg. The total body clearance (Cl_B) was calculated 0.54±0.01 L.kg^{-1}.h^{-1}. The maximum plasma concentration of lambda-cyhalothrin (C_{max}) was noted to be 6.42±0.11 µg.ml^{-1} at t_{max} of 2.00 ± 0.00 h.

Toxicokinetic Parameters in Various Organs

The mean extrapolated zero time concentration of lambda-cyhalothrin in tissue of various organs liver, kidney, heart, adrenal, spleen, brain, testis and ovaries during absorption phase (A') were calculated 28.5±3.67, 8.12±3.97, 45.5±7.41, 68.5±4.63, 111.3±11.8, 31.1±2.63, 2.27±0.19, 36.4±16.5 and 0.93±0.04 µg/ml, respectively. Whereas, the mean extrapolated zero time concentration of lambda-cyhalothrin in tissue of various organs liver, kidney, heart, adrenal, spleen, brain, testis and ovaries during elimination phase (B) were noted to be 5.96±0.46, 0.93±0.04, 0.98±0.06, 2.36±0.34, 1.69±0.09, 4.25±0.14, 0.63±0.02, 0.26±0.01 and 2.30±0.76 µg/ml, respectively (Table 2). The highest mean value of absorption rate constant (Ka) was observed in brain tissue 3.57±0.08, whereas lowest value of absorption rate constant (Ka) was observed in ovaries 0.74±0.36 h^{-1} respectively. The mean elimination rate constant (β) in tissue of various organs liver, kidney, heart, adrenal, spleen, brain, testis and ovaries were observed 0.15 ± 0.01, 0.18±0.001, 0.18±0.01, 0.16±0.01, 0.16±0.003, 0.06±0.01, 0.13±0.01 and 0.10±0.02 h^{-1}, respectively.

The mean absorption half-life ($t_{½Ka}$) in tissue of various organs were calculated to be 0.24±0.02 (liver), 0.33±0.013 (kidney), 0.54±0.03 (heart), 0.38±0.06 (adrenal), 0.28±0.01 (spleen), 0.31±0.11 (brain), 0.29±0.03 (testis), 5.34±4.68 (ovaries) whereas, elimination half-

life ($t_{½\ \beta}$) were calculated to be 4.58±0.23 (liver), 4.29±0.29 (kidney), 4.04±0.27 (heart), 4.39±0.21 (adrenal), 0.16±0.003 (spleen), 4.46±0.26 (brain), 5.65±0.47 (testis) and 7.48±1.65 (ovaries) h. The mean area under curve (AUC in tissue of various organs in were calculated to be 16.4±0.27 (liver), 6.33±0.012 (kidney), 17.7±0.57 (heart), 14.0±0.91 (adrenal), 44.9±0.65 (spleen), 7.50±0.14 (brain), 3.20±0.12 (testis), 50.0±1.73 (ovaries) $\mu g.ml^{-1}.h^{2}$ and mean area under first moment curve (AUMC) were noted to be 51.1±3.22 (liver), 39.8±2.35 (kidney), 72.8±12.8 (heart), 70.6±3.81 (adrenal), 170.8±5.02 (spleen), 27.0±2.11 (brain), 50.6±2.11 (testis), 327.3.0±30.5 (ovaries) $\mu g.ml^{-1}.h^{2}$ respectively with the mean residence time (MRT) 3.10±0.16 (liver), 6.28±0.26 (kidney), 4.92±0.09 (heart), 5.08±0.30 (adrenal), 3.78±0.06 (spleen), 3.57±0.22 (brain), 5.50±0.44 (testis), 6.33±0.29 (ovaries) h. The highest mean value of volume of distribution (Vd_{area}) was calculated in testis 50.6±2.11 and lowest in spleen 2.69±0.06 L kg^{-1}. The mean value of total body clearance (Cl_B) highest in testis 6.28±0.22 whereas lowest in ovaries 0.39±0.02 $L.kg^{-1}.h^{-1}$. The maximum tissue concentration of lambda-cyhalothrin (C_{max}) in various tissue was noted to be 7.75±0.28 (liver), 1.43±0.02 (kidney), 4.90±0.42 (heart), 4.87±0.69 (adrenal), 17.9±0.38 (spleen), 6.63±0.25 (brain), 1.46±0.10 (testis), 9.77±0.81 (ovaries) $\mu g.ml^{-1}$ at t_{max} of 1.00±0.00 (liver), 1.00±0.00 (kidney), 2.00±0.00 (heart), 1.00±0.00(adrenal), 1.00±0.00 (spleen), 0.50±0.00 (brain), 0.50±0.00 (testis), 2.00±0.00 (ovaries) min.

Table 1-Toxicokinetic parameters of lambda-cyhalothrin (20 mg/kg) in serum following oral administration in healthy wistar albino rats.

Parameter	Unit	1	2	3	4	5	6	Mean±S.E.M
A’	$\mu g.ml^{-1}$	4.71	4.72	12.88	4.29	3.19	3.09	5.48±1.51
K_a	h^{-1}	0.50	0.519	1.578	0.428	0.156	0.250	0.57±0.21
$t_{½Ka}$	h	1.39	1.34	0.44	1.62	4.44	2.78	2.00±0.47
B	$\mu g.ml^{-1}$	5.98	6.21	7.86	5.97	5.19	4.55	5.96±0.46
β	h^{-1}	0.144	0.139	0.153	0.132	0.130	0.123	0.14±0.004
$t_{½\ \beta}$	h	4.820	4.977	4.529	5.240	5.335	5.651	5.09±0.16
AUC	$\mu g.ml^{-1}.h$	35.8	38.4	36.6	38.6	37.1	37.3	37.3±0.44
AUMC	$\mu g.ml^{-1}.h^{2}$	250.2	277.1	259.4	291.9	274.4	283.7	272.8±6.31
$Vd_{(area)}$	$l.kg^{-1}$	3.89	3.75	3.57	3.91	4.15	4.37	3.94±0.12
Cl_B	$l.kg^{-1}.h^{-1}$	0.56	0.52	0.55	0.52	0.54	0.54	0.54±0.01
MRT	h	7.0	7.2	7.1	7.6	7.4	7.6	7.32±0.11
C_{max}	$\mu g.ml^{-1}$	6.3	6.5	6.1	6.6	6.8	6.2	6.42±0.11
t_{max}	h	2.0	2.0	2.0	2.0	2.0	2.0	2.00±0.00

Table-2 Toxicokinetic parameters of lambda-cyhalothrin (20 mg/kg) in various organs following oral administration in healthy wistar albino rats.

Parameter	Unit	Toxicokinetics parameters in different organs							
		Liver	Kidney	Heart	Adrenal	Spleen	Brain	Testis	Ovaries
A'	$\mu g.ml^{-1}$	28.5±3.67	8.12±3.97	45.5±7.41	23.66±6.34	111.3±11.8	31.1±2.63	2.27±0.19	36.4±16.5
K_a	h^{-1}	2.15±0.09	2.82±0.13	1.31±0.08	2.30±0.62	2.44±0.014	3.57±0.08	2.55±0.33	0.74±0.36
$t_{½Ka}$	h	0.33±0.013	0.24±0.02	0.54±0.03	0.38±0.06	0.28±0.01	0.31±0.11	0.29±0.03	5.34±4.68
B	$\mu g.ml^{-1}$	0.93±0.04	0.98±0.06	2.36±0.34	1.69±0.09	4.25±0.14	0.63±0.02	0.26±0.011	2.30±0.76
β	h^{-1}	0.15±0.01	0.18±0.001	0.18±0.011	0.16±0.01	0.16±0.003	0.16±0.01	0.13±0.01	0.10±0.02
$t_{½\,\beta}$	h	4.58±0.23	4.29±0.29	4.04±0.27	4.39±0.21	4.20±0.08	4.46±0.26	5.65±0.47	7.48±1.65
AUC	$\mu g.ml^{-1}.h$	16.4±0.27	6.33±0.12	17.7±0.57	14.0±0.91	44.9±0.65	7.50±0.14	3.20±0.12	50.0±1.73
AUMC	$\mu g.ml^{-1}.h^2$	51.1±3.22	39.8±2.35	72.8±12.8	70.6±3.81	170.8±5.02	27.0±2.11	17.8±2.09	327.3±30.5
$Vd_{(area)}$	$l.kg^{-1}$	8.04±0.34	19.5±0.98	6.60±0.35	9.26±0.81	2.69±0.06	17.1±0.88	50.6±2.11	4.15±0.71
Cl_B	$l.kg^{-1}.h^{-1}$	1.22±0.02	3.17±0.06	1.14±0.04	1.46±0.10	0.44±0.08	2.66±0.06	6.28±0.22	0.39±0.02
MRT	h	3.10±0.16	6.28±0.26	4.92±0.09	5.08±0.30	3.78±0.06	3.57±0.22	5.50±0.44	6.33± 0.29
C_{max}	$\mu g.ml^{-1}$	7.75±0.28	1.43±0.02	4.90±0.42	4.87±0.69	17.9±0.38	6.63±0.25	1.46±0.10	9.77±0.81
t_{max}	h	1.0±0.00	1.0±0.00	2.0±0.00	1.0±0.00	1.00±0.00	0.50±0.00	0.5±0.00	2.00±0.00

IMMUNE RESPONSE

Tube Agglutination Test: The antibodies titre in rat against *Salmonella typhimurium* 'O' antigen significant ($p<0.01$) reduced antibody titre as compared to control (Table 3).

Table 3 Effect of lambda-cyhalothrin (10 mg/kg) on humoral immune response in rat induced by *Salmonella typhimurium* 'O' antigen by tube agglutination test.

Groups/Treatment	Antibody Titre (Mean±SE)
Vehicle control	80.00±8.43
Lambd-acyhalothrin (10 mg/kg)	16.67±4.01**

Data with superscripts* in a column differ significantly ($P<0.01$)

Ex Vivo and *In Vitro* Effects of Lambda-Cyhalothrin on Splenocytes Proliferation Assay

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 4 and Plate 1 & 2. 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.

Table 4. 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± 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 ($P<0.05$)

i) 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 5 and Plate 3 & 4. 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.

Table 5. 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 ($P>0.05$)

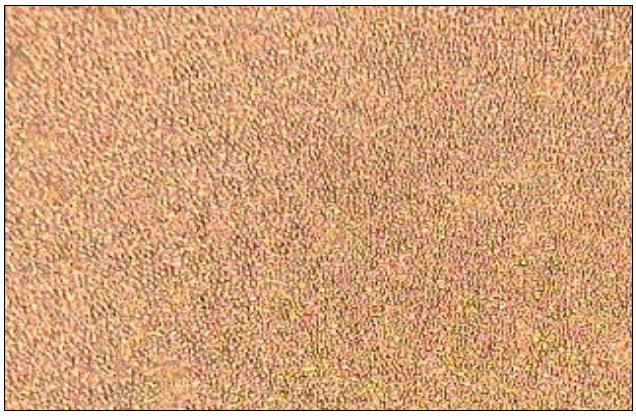

Plate 1 Ex-vivo splenocytes proliferation assay showing stimulated splenocytes in control group.

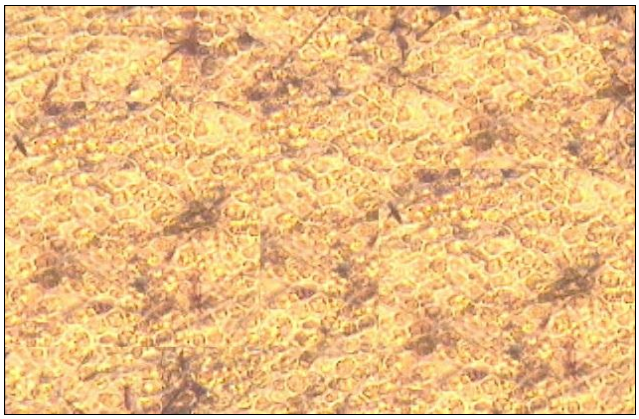

Plate 2 Ex-vivo splenocytes proliferation assay showing decrease in splenocytes density and formazone crystals formed within the splenocytes in lambda-cyhalothrin treated group

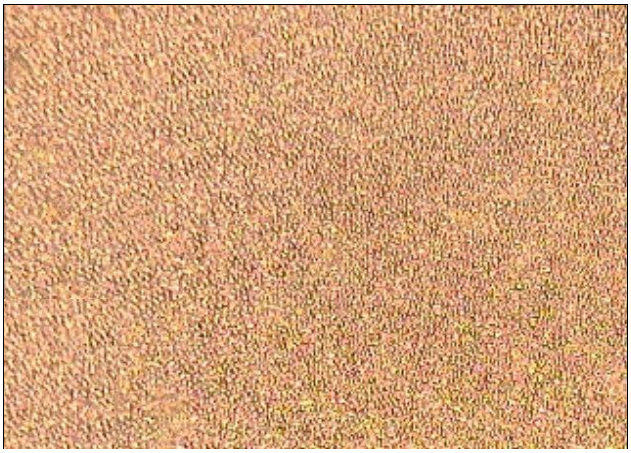

Plate 3 In-vitro splenocytes proliferation assay showing stimulated splenocytes in control group

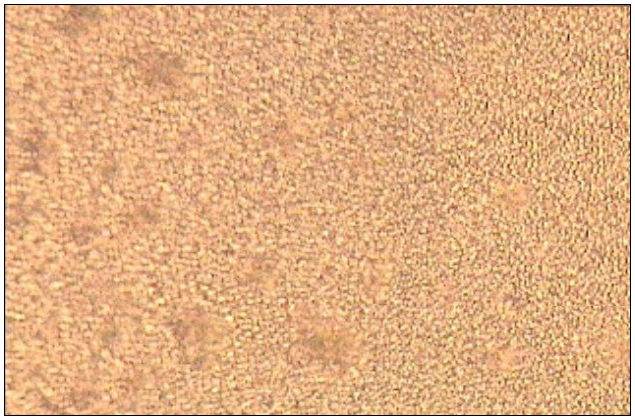

Plate 4 In-vitro splenocytes proliferation assay showing decreased stimulated splenocytes in lambda-cyhalothrin treated group

DISCUSSION

The toxicokinetics characteristics combined with toxicodynamic patterns must be considered in the use safety evaluation of lambda-cyhalothrin. In present study was undertaken to estimation of lambda-cyhalothrin in biological fluid and tissue in order to evaluate its pharmacokinetics. In the study reported here the kinetics of lambda-cyhalothrin after a single oral (20 mg kg^{-1}) administration was determined in wistar albino rats. The disposition of lambda-cyhalothrin after oral administration in rats was best described by use of a one-compartment model. Lambda-cyhalothrin absorbed rapidly from the GI tract of fasted adult rats and peak blood levels were manifest within 2h which is close agreement with data reported for deltamethrin [14]. Disappearance of lambda-cyhalothrin from plasma and tissues of rats were characterized by an initial rapid distribution phase followed by a slower elimination phase. The $T_{1/2}$ of lambda-cyhalothrin (5.09±0.16hafter oral administration) was shorter to that observed [15].

The values of $Vd_{(area)}$ 3.94 $L.kg^{-1}$ indicate that lambda-cyhalothrin easily penetrated all tissues, in agreement with data reported also for the pyrethroids permethrin and deltamethrin [16, 17]. Lambda-cyhalothrin was extensively absorbed after oral administration and maximal plasma concentration C_{max} (6.42µg.ml^{-1}) at t_{max} 2.00 h which is greater to those of study in which deltamethrin administered at a comparable dose of 26 mg kg^{-1} yield a C_{max} of 0.46 µg.ml^{-1} at t_{max} 1-2 h after oral administration [17]. The mean residence time (MRT) of lambda-cyhalothrin in blood was observed 7.32 h which is close agreement with the observation [15].

Although the symptoms of toxicity of lambda-cyhalothrin were not quantitated in the present study, the administration of lambda-cyhalothrin to rats at oral (20 mg. kg^{-1}) dose produced slight whole body tremor, sign of toxicity typical of type II pyrethroids [18, 19]. The symptoms

were observed 1–3 h and 10–15 min after oral administration, respectively. Complete recovery was not observed until 12 h after oral dosing. Lambda-cyhalothrin rapidly enters the brain C_{max} 6.63 µg/ml at t_{max} 0.50 h. lambda-cyhalothrin also achieved high concentration in tissue of organs liver C_{max} 7.75, spleen C_{max} 17.9 and ovaries 9.77 µg/ml at t_{max}1.0, 1.0 and 2.0 h, respectively as compared to plasma C_{max} 6.42 µg/ml. The mean residual time of lambda-cyhalothrin was observed lowest in liver 3.10 h and brain 3.57 h means lambda-cyhalothrin rapidly cleared from brain and liver tissue. Whereas, slowly cleared from ovaries followed by kidney means lambda-cyhalothrin spend more time in ovaries and kidneys. Lambda-cyhalothrin rapidly cleared from testis (Cl_B 6.28 $l.kg^{-1}.h^{-1}$) followed by kidney and brain Cl_B 3.17 $l.kg^{-1}.h^{-1}$ and Cl_B 2.66 $l.kg^{-1}.h^{-1}$, respectively. The humoral immune response was evaluated by measuring antibody titre in rats against *Salmonella typhimurium* 'O' antigen by tube agglutination test. The results indicated that lambda-cyhalothrin suppress antibody titre against *Salmonella typhimurium* 'O' antigen as compared to negative control. The recorded reduction of humoral immune response confirmed the immunossupression occurred in rats after exposure to different type II pyrethroids [20, 21, 22]. 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 [23]. This assumption was ascertained by the previously established lowering effect of the insecticide on the antibody titers in sera of treated rats. The result shows depletion of splenocytes exhibit the cytotoxicity of lambda- cyhalothrin. Similar results in which marked thymocyte depletion were previously obtained in rats exposed to cypermethrin [20]. Moreover, high doses of cypermethrin, supercypermethrin forte and deltamethrin, displayed an immunosuppressive effect on cell-mediated immune response in adult mice, rats and goats [5, 20, 21, 24, 25]. Also, a marked lymphocyte depletion was observed in the thymus and lymph nodes of cypermethrin–treated rats [20, 24].

CONCLUSION

In conclusion, toxicokinetics effect of lambda-cyhalothrin on exposure at the tested doses suggests that sub-acute and/or chronic exposure to lambda cyhalothrin in the environment has the potential to produce neurotoxic as well as neurotoxic effect. Lambd-acyhalothrin primarily affects nervous tissue and spleen. 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 toxic effects.

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