Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3 (1): 322-325

Received: 07-12-2014
Accepted: 09-01-2015

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In vitro and *in silico* effect of λ- Cyhalothrin on serum lactate dehydrogenase in *Rattus norvegicus*

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Abstract
λ- Cyhalothrin belongs to a group of pyrethroid pesticides being used in various pest control programs. Prediction of binding of λ- Cyhalothrin on LDH in *Rattus norvegicus* was analyzed in the current study, using *in silico* and *in vitro* methods. The Molegro Virtual Docker (MVD) was used for *in silico* study and diagnostic reagent kit for *in vitro* study. The comparative mole dock score, root-mean square deviation (RMSD), affinity, interacting residues of LDH, number of hydrogen bond interaction, docking score, protein steric interaction energy (Protein EvdW) and the interaction of residues were evaluated with the help of *in silico* study. The λ- Cyhalothrin binds with the lysine (Lys56) and tyrosine (Tyr 246) and forms three hydrogen bonds with LDH. λ- Cyhalothrin causes dose as well as time dependent toxicity after acute and late sub chronic treatments has been proved after *in vitro* study.

Keywords: λ- Cyhalothrin, Toxicity, *In vitro*, *In silico*, Lactate dehydrogenase, MVD, Lysine, Tyrosine.

1. Introduction
Pesticides are used against various pests for human welfare but are also damage to humans as they eventually find their route to the human body via food chain and induce damage [1-2]. Blood can be used as an important diagnostic tool to investigate the toxicity of any foreign component inside the body including pyrethroid pesticides. λ -cyhalothrin is an insecticide [3] and highly toxic to fish [4], disrupts the nervous system of insects causing paralysis or even death [5], decreases body weight gain and alters blood chemistry in rats, especially an increased frequency in mammary tumors in female mice [6] and may cause dizziness, headache, nausea, lack of appetite, fatigue, seizures and coma in humans after severe poisonings of λ–cyhalothrin [7]. It is a well-known fact that exchange of material in living tissue is done through blood and, therefore, the alteration of any component of blood can be assessed by evaluating serum biochemistry [8-9]. The aim of the present study was to evaluate the effect of λ -cyhalothrin using *in vitro* and *in silico* methods in albino rats by the estimation of serum lactate dehydrogenase (LDH). It can be anticipated that the changes induced by λ- Cyhalothrin in the albino rat, can serve as an indicator for similar effects on other allied and higher mammalian species.

2. Materials and Methods
The present study was conducted with adult individuals of albino rats *Rattus norvegicus* of almost equal size and weight, representing both the sexes were selected randomly from inbred colony from July to September 2014 and acclimatized for two weeks to laboratory conditions prior to dose administration. The experimental and control rats were maintained under standard conditions of light and temperature, were provided standard pellet diet (gold mohar lab animal feeds) and water *ad libitum*. The rats were weighed and kept in four sets namely, one acute, one sub chronic and two control of 5, 20,5,20 rats respectively. Technical grade λ - Cyhalothrin (RS)-α cyno-3phenoxybenzyl (Z)-(1RS)-cis,-3-(2chloro-3, 3, 3-trifluoropropenyl) 2,2-dimethylcyclopropanecarboxylate) was used as experimental compound (Fig- 1).The doses for acute and sub-chromic studies were 757 mg/kg. bwt. and 38 mg/kg b.wt respectively on the basis of LD50 [10].

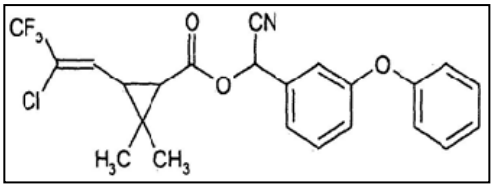

Fig 1: Structure of λ – Cyhalothrin

The rats of control set for acute and sub chronic were given ground nut oil as a vehicle. Rats in each set were taken out after 24 hours from acutely dosed rats and on the 7th, 14th and 21st day from sub chronically treated animals and were anaesthetized with chloroform. The blood samples were collected using sterilized needles into plain vials, kept for 30 minutes at room temperature and centrifuged at 3000 rpm for 20 minutes to separate out the serum after predetermined time intervals from control and treated groups. The estimation of Lactate dehydrogenase (LDH) was done by using the diagnostic reagent kit for *in vitro* determination of the activity of LDH in serum, manufactured by Span diagnostics Ltd. India. Statistical significance between experimental and control values were calculated according to Fisher's student 't' test [11].

For *in silico* investigation three dimensional X-ray crystallized structure of lactate dehydrogenase (PDB: 4AJ1) was downloaded from the protein data bank [12]. The downloaded protein has four chains A, B, C & D with 331 residues (Fig 2). It was taken as receptor protein and most suitable site was predicted by using q site finder ligand binding site prediction tool (http://www.modelling.leeds.ac.uk). Because of the priority of site, lactate dehydrogenase has been selected for docking with ligand λ- Cyhalothrin (CID_71464055). The synthetic pyrethroid pesticide was downloaded from Pub Chem Compound (http://www.ncbi.nlm.nih.gov).The docking simulation was performed by using docking software Molegro Virtual Docker (MVD) for the selected pesticide (ligand) and lactate dehydrogenase (protein). It shows mole dock score, RMSD, affinity (the estimated binding affinity in kj /mol), docking score, protein EvdW and interacting interaction (the interaction energy among the pose and the cofactor), number of H-bond and interaction between interacting residues of receptor human oxyhaemoglobin, which indicates towards the formation of stable complexes among ligand and the receptor molecule[13]. MVD visualizer was used for interaction site prediction.

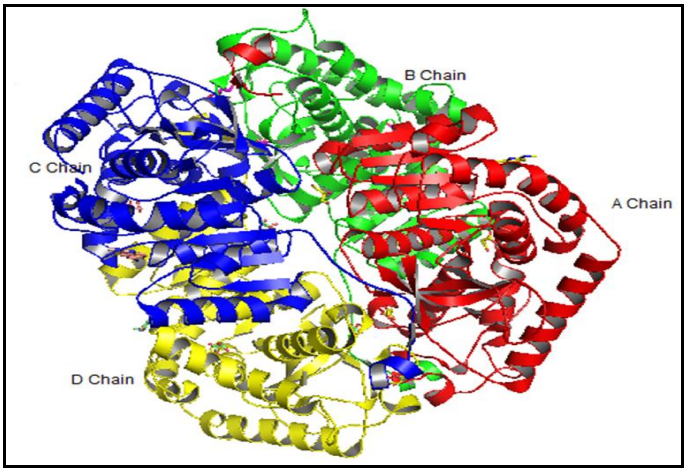

Fig 2: Structure of Lactate dehydrogenase

3. Results & Discussion

In vitro results are tabulated in Table-1. The serum lactate dehydrogenase (LDH) was significantly increased after λ-Cyhalothrin intoxication in comparison to control values. The increase was significant after one day and late sub chronic 14, 21 days treatments while; increase was non significant after 7days sub chronic treatment (Table-1). Elevation in serum level of LDH is an outcome of tissue necrosis, particularly of the liver [14]. Further, λ - Cyhalothrin is also known to cause haemolytic anemia, resulting in lysis of the red blood cell membrane, which again causes enhanced LDH levels in the serum of pyrethroid intoxicated rats [15-16]. However, it has been interpreted that in sub chronic treatment raised LDH activity in the serum of albino rat after cypermethrin treatment may result from an increase in LDH synthesis followed by its leakage into the blood [17]. The same appears to be true after λ-Cyhalothrin intoxication in the present study. Increased LDH activity reflects that acute and sub chronic dose of λ-Cyhalothrin exert cytotoxic and haemolytic effect [18].

Table 1: Effect of λ - Cyhalothrin on Serum Lactate dehydrogenase (lU/100 mlLDH) in *Rattus norvegicus* after Acute and Sub Chronic Treatments

S. No	Treatment Duration(in days)	Dose (mg/kg b.wt.)	Range	Mean±SE
1.	Control º (1day)	-	45.0-55.0	51.000±3.055
2.	Acute (1day)	757	64.5- 69.8	67.150±2.650*
3.	Control º (7 days)		51.5 - 54.7	52.90 ±0.945
4.	S-Ch,(7days)	38	52.5 -68.5	60.500±8.000NS
5.	Control º (14 days)	-	43.5-54.5	49.267±3.187
6.	S-Ch (14 days)	38	67.0-75.0	71.000±4.000*
7.	Controlº(21 days)	-	45.0-53.0	49.333±2.333
8.	S-Ch (21 days)	38	74.0-87.5	80.750±6.750*

S-Ch = Sub chronic, º =controls were given the same quantity of diluents, * = p<0.05,

The *in silico* results obtained are tabulated in (Table- 2) and these results also support the *in vitro* results. The interaction analysis of binding of λ- Cyhalothrin with serum lactate dehydrogenase (LDH) has been done to find out the residues that are involved in binding. MVD and its visualizer were used for interaction site analysis [19]. The λ- Cyhalothrin shows very high affinity to bind with serum lactate dehydrogenase (LDH) and its A-chain interacts with Trp249, Tyr 246, Arg98 and B-chain with Lys56, Tyr 246, Gly 28, Ala29, Met 32, Glu 60, Arg 98. The λ- Cyhalothrin forms 3 hydrogen bonds with Tyr 246, Lys56 and Tyr 246 (Table-3, Fig -3).

Table 2: Comparative Docking Simulation Result of λ Cyhalothrin Pesticide with Lactate dehydrogenase (PDB: 4AJ1) using MVD

S. No.	Mole Dock Score	RMSD	Affinity	Intracting	Docking Score
1	-95.3058	7.11266	-3.3974	-04.986	-96.6525
2.	-94.5949	9.21627	-44.731	-113.599	-94.6443
3.	-90.4278	9.02482	-43.5345	-104.593	-94.1814
4.	-93.4277	9.5146	-43.6404	-103.8388	-92.837
5.	-85.8021	10.2114	-41.0363	-100.302	-87.1149

Table 3: Lactate dehydrogenase (PDB: 4AJ1) protein residues interact with selected λ Cyhalothrin pesticide using MVD (Highlighted residues are involved in H-bonding interaction with ligands)

S. No.	ligand	Interacting residues of receptor Human oxyhaemoglobin	No. of H-bond interaction
1.	**Cyhalothrin**	**Tyr 246**, **Lys56**, **Tyr 246,** Trp249, Gly 28, Ala29, Met 32, Glu 60, Arg98, Arg 98,	03

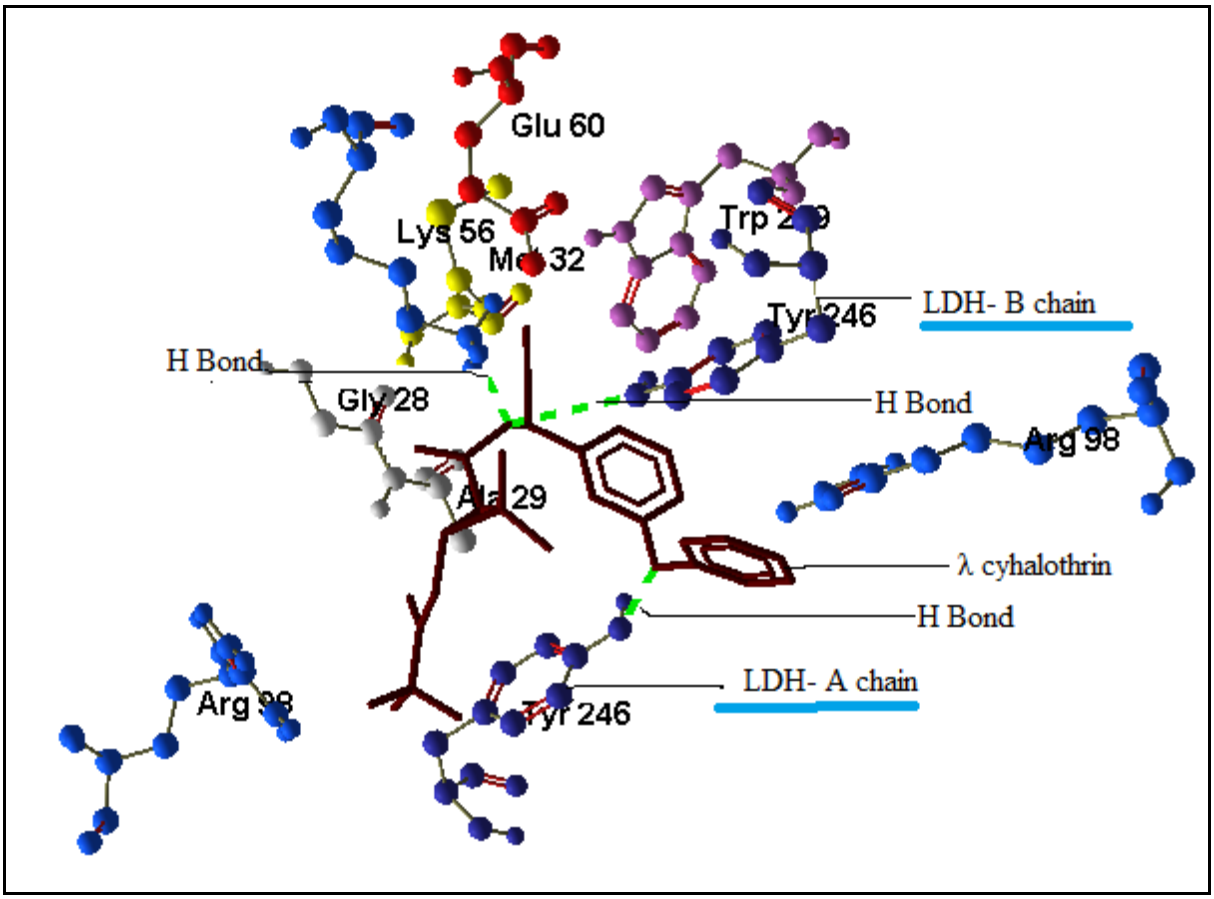

Fig 3: Docked conformation of hydrogen bonding view of λ Cyhalothrin with interacting Lactate dehydrogenase at the active site cavity

Computational methods can turn out to be very useful for comparing *in vitro* results and *in silico* results. Docking study shows that amino acid residue i.e, Lys 56 and Tyr 246 bind with λ- Cyhalothrin forming 3 hydrogen bonds (Fig -3). Amino acids are simply, monomeric subunits that provide the key to the structure of thousands of different proteins. They are composed of a carboxyl group and an amino group, bound to a carbon atom [20]. Lysine (Lys) contains a positively charged amino acid on its side-chain which is sometimes involved in forming hydrogen bonds with negatively charged non-protein atoms [21]. Similarly tyrosine (Tyr) is a non-essential amino acid with a polar side group. Alterations in the metabolism of amino acids and proteins with neoplasms are usually the consequence of certain dysfunctions in the host metabolism, such as accelerated gluconeogenesis and increased protein synthesis by the liver [22-23]. Tyrosine kinases activate numerous signaling pathways, leading to cell proliferation, differentiation, migration, and metabolic changes [24]. Moreover, enhanced tyrosine kinase activity is the hallmark of a sizable fraction of cancers as well as other proliferative diseases due to pesticide toxicity [25-26].

4. Conclusion

Consequently, *in vitro* results support *in silico* results in the present study. λ- Cyhalothrin binds with amino acids of LDH and forms 3 hydrogen bonds which disturb the normal functioning of the serum LDH. The marked increase in LDH after acute and late sub chronic treatments may be due to damage of hepatic tissue as a response by the body system towards overcoming stress induced by the test compound. The uncontrolled use of insecticide deserves re-evaluation to establish its safety indices.

5. Acknowledgement

I am thankful to UGC, Delhi for providing the financial assistance and C. S. J. M. University, Kanpur, India for providing lab facilities.

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