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Effect of λ-cyhalothrin on Redox Homeostasis and Neuropharmacological Activities in Wistar Rats

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Abstract

The present study was carried out to assess the behavioral, neurobiochemical changes following λ-cyhalothrin exposure and to evaluate the neuropharmacological activity in in Wistar rats using well defined animal models. Neurobehavioral assessment was carried as per modified Irwin test. To determine the oxidative profile of brain tissue, rats were randomly divided into two groups consisting of six rats each. Group I served as vehicle-treated control and Group II received λ-cyhalothrin at 7.8 mg/kg (*per os*) for a period of 7 days. The neuropharmacological studies were carried out in rats following administration of λ-cyhalothrin at a dose rate (*per os*) of either 1/5th or 1/10th LD50. Neurobehavioral assessment showed prominent autonomic sign(s) followed by depression following single administration of λ-cyhalothrin (1/10th LD50). Short-term exposure to λ-cyhalothrin-induced lipid peroxidation as evident by significant increase in malondialdehyde levels in the brain tissue. Marked alteration of antioxidant defense mechanism(s) in the brain tissue was observed by the reduced activity of superoxide dismutase and catalase. Further, the tissue glutathione (GSH) (reduced; GSH) content was also significantly ($P < 0.05$) depleted. λ-cyhalothrin exerted a significant reduction in spontaneous motor activity as well as forced motor activity in dose-dependent manner, thus demonstrated reduced activity of central nervous system. Interestingly, λ-cyhalothrin exerted nociceptive action, which was comparable to control group. Further, there was marked increase in the duration of strychnine-induced (2 mg/kg; i.p.) tonic-clonic convulsions in rats. Overall, short-term exposure to sub lethal dose of λ-cyhalothrin causes disturbances in brain redox homeostasis and alters neuropharmacological activity in Wistar rats.

Keywords: Neuropharmacology, Oxidative Stress, Wistar Rats, λ-Cyhalothrin

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1. Introduction

It has been well documented that among the several pesticides those having Central Nervous System (CNS), disruptive properties are known to adversely impair nervous system function under laboratory, field, clinical, and occupational settings. In India, 51% of the food commodities contaminated with chemical residues out of which 20% contributed by pesticides alone.[1] Synthetic pyrethroids accounts for approximately one-fourth of world insecticide market. They are increasingly being used in agriculture, veterinary applications on farm and pet animals, for the protection of stored foodstuffs, for the control of endemics and parasites in public health programs as well as for household applications to control cockroaches, flies, mosquitoes, and ticks. λ-cyhalothrin (alpha-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2 dimethyl-cyclopropane-carboxylate), a type II cyano group pyrethroid is one of the major insecticide used today in plant protection programs. It is used as a stomach and contact insecticide having ovicidal and miticidal properties.

Toxicokinetic studies on λ-cyhalothrin revealed that it accumulates in nervous tissue including neuromuscular fibers.[2] Extensive application of pesticides is usually accompanied with serious problems of pollution and

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health hazards. Several studies confirmed that the CNS is the principle target of synthetic pyrethroids.[3,4]

λ-cyhalothrin accumulates in neuronal tissue by virtue of its lipophilicity and known to induce oxidative stress and disrupt the neuronal integrity.[5] Further, λ-cyhalothrin interfere with several membrane bound ionic channels such as voltage-gated Na^{+2} (VGSC) and $GABA_A$ linked Cl^- channels.[6] Further, the type II pyrethroids can stimulate Ca^{+2} influx and favor transient (outward) K^+ current amplitude (VGKC) in neuronal tissues.[7] The mechanisms by which λ-cyhalothrin modulate the neuropharmacological activity are not clear. Furthermore, information available on the oxidative mechanism of brain dysfunction is meager. Thus, it is hypothesized that impairment in neuronal (brain) function on account of peroxidative stress by xenobiotics such as λ-cyhalothrin would influence or modulate neuropharmacological activity. In the current study, we evaluated the oxidative stress profile in brain tissues following short-term exposure to λ-cyhalothrin and evaluated its influences on λ-cyhalothrin ($1/5^{th}$ or $1/10^{th}$ LD_{50}) on certain neuropharmacological paradigms viz. behavioral analysis, spontaneous motor and forced locomotor activity, CNS-mediated analgesia, and strychnine-induced convulsions in Wistar rats.

2. Materials and Methods

2.1 Chemicals

Strychnine sulfate from M/s Sigma Chemicals, St. Lois (USA). Diazepam hydrochloride (Ranbaxy Pharma, New Delhi) and λ-cyhalothrin (Insecticide India Ltd., Jammu and Kashmir) were obtained. All other chemicals manufactured by M/s SRL Chemicals (India) or Hi Media Chemicals Ltd., (India) were employed.

2.2 Experimental Design

All the following experimental procedures were performed in Wistar rats. The animals were maintained (28–31°C temperature, 50–55 % relative humidity) in polypropylene cages and provided with feed and water *ad libitum*. Twelve hours before undertaking each of the following experiment, the animals had access to water, but not feed. The dosage of λ-cyhalothrin employed in the various experiments was either $1/10^{th}$ and/or $1/5^{th}$ of the reported oral LD_{50} value of λ-cyhalothrin in rats (78 mg/kg in rats).[8] Normal saline (0.9% w/v NaCl) was used in rats belonging to control groups.

For biochemical analysis, rats were randomly divided into two groups consisting of six rats in each. Group I served as vehicle-treated (normal saline) control and Group II received λ-cyhalothrin at 7.8 mg/kg (*per os*) for a period of 7 days. On the 8^{th} day, animals were scarified by cervical dislocation. Further, brain tissue was collected and blotted with tissue paper, then weighed, and kept at −80°C until analysis.

A 50 mg of brain was taken in 0.5 ml of ice-cold phosphate buffer saline (pH 7.4). Another 20 mg of brain was taken separately in 0.2 ml of 0.02 M ethylenediaminetetraacetic acid for estimation of reduced glutathione (GSH). The homogenate (10%) was prepared with a homogenizer under ice-cold condition and centrifuged for 10 min at 3000 rpm. The supernatant was stored at −20°C until subjected to assay.

3. General Behavior

For assessing the general behavior of rats, $1/10^{th}$ (7.8 mg/kg) of reported LD_{50} λ-cyhalothrin was administered *per os* to Wistar rats ($n = 6$). Autonomic, motor, sedation, and stereotypy activity were recorded according to modified Irwin test.[9] Rats were scored as +++ when they exhibited aggression and fighting behavior, while rats showing as "0" for normal social behavior were allotted "0" score.

4. Assessment of Brain Peroxidative Damage

Brain peroxidative damage was assessed by evaluating the lipid peroxidation (LPO) in terms of malondialdehyde (MDA) production as described by Paula *et al.*[10]

5. Determination of Brain-Reduced Glutathione Content

Reduced GSH content was measured in the brain homogenate by the method of Sedlak and Lindsay.[11]

6. Determination of Brain Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity in the brain was determined by the procedure of Madesh and Balasubramanian.[12]

7. Determination of Brain Catalase Activity

Catalase activity in the brain homogenate was assayed by the spectrophotometric method of Aebi.[13]

8. Protein Estimation

The protein content in the supernatant of the brain homogenate was determined following the method of Lowry *et al.*[14] using bovine serum albumin as the standard.

9. Spontaneous Motor Activity

The spontaneous motor activity was measured using actophotometer (Inco Instruments, Ambala, India) following the method of Kulkarni.[15] The movement of the animal cuts off a beam of light falling on the photocell and a count was recorded and displayed digitally. The Wistar rats were divided into three groups of six each. Each rat was placed individually in the actophotometer and the basal activity was measured. Animals in Group I were administered (*per os*) with distilled water and Groups II and III with λ-cyhalothrin 7.8 and 15.6 mg/kg body weight, respectively. To measure locomotor activity, number of squares crossed by the animal every 30 min during 120 min of experimental period was counted.

10. Forced Locomotor Activity

The effect of λ-cyhalothrin on forced locomotor activity as a measure of skeletal muscle tone and coordinated movements of animals was studied using rotarod (Rotarod 4C, MKM, Chennai, India) as described by Dunham and Miya[16] in rats. The rats which were trained to remain on the horizontal rod rotating at 20 rpm were selected for the study. Only those rats which have demonstrated their ability to remain on the rotating rod for at least 2 min were selected and used for the experiment. Forced locomotor activity was observed 30, 60, and 90 min after treatment in Group I (control) and Groups II and III administered (*per os*) with λ-cyhalothrin at 7.8 and 15.6 mg/kg body weight, respectively. After administration of λ-cyhalothrin, rats were placed on the rotating rod and the "fall off" time for 5 min was recorded at regular intervals of 30, 60, and 90 min after the treatments. The "fall off" time (s) after the treatment was considered for evaluation and those rats which fell from the rotating rod within 2 min were considered "affected".

11. Analgesic Activity

Eddy's hot plate method described by Turner[17] was used to determine the analgesic activity in Wistar rats. The animals were dropped gently on a plate maintained at 55 ± 5°C. Reaction time was taken as the interval between the instant the animal reaches the hot plate till the animal shows acute discomforts such as licking of forepaws, kicking with hind legs, and jumping or frisking of the enclosure. Measurements were carried out after 30, 60, and 90 min after oral administration of λ-cyhalothrin (7.8 and 15.6 mg/kg). The control group was given normal saline (0.5 ml, *per os*). Analgesic activity was considered to be adequate if the reaction time crossed 15 s in treated rats.

12. Strychnine-Induced Convulsions

Rats were randomly allotted into three groups. Control (Group I) rats were administered with strychnine (2 mg/kg; i.p.) 30 min after normal saline (*per os*). Positive control group of rats (Group II) received strychnine (2 mg/kg; i.p.) 15 min after diazepam hydrochloride (3 mg/kg; i.p.) apart from receiving normal saline (*per os*). Animals in Group III were administered with similar dose of strychnine 15 min after *per os* administration of λ-cyhalothrin (7.8 mg/kg). The time required for onset action and tonic-clonic convulsions were recorded,[18] and mortality, if any, was observed up to ½ h following experiment. Any rat that did not convulse within 30 min after strychnine administration was considered as "protected."

13. Data Analysis

Data have been expressed as mean ± standard error with "*n*" equal number of animals. Data of oxidative stress were analyzed by Student's *t*-test and all other data were analyzed by one-way ANOVA followed by Duncan's

multiple comparison *post-hoc* test using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Difference at $P < 0.05$ was considered statistically significant.

14. Results

Neurobehavioral assessment was carried as per modified Irwin test. In the present study, oral administration of λ-cyhalothrin at the dose rate of 7.8 mg/kg in rats shown prominent autonomic, motor sign(s) followed by depression [Table 1].

Effects of short-term exposure (*per os*) to λ-cyhalothrin on LPO level (nmol MDA/g tissue) in rat brain are presented in Figure 1a. Exposure to λ-cyhalothrin increased LPO significantly ($P < 0.05$) by 38% compared to the control level. Figure 1b illustrates the effects of λ-cyhalothrin on the GSH antioxidative system. Short-term exposure to λ-cyhalothrin decreased the brain GSH content (mmol of GSH/g tissue) significantly by 41% over the control.

Effects of λ-cyhalothrin on SOD activity (unit/mg protein) and catalase activity (mmol H_2O_2 utilized/min/mg protein) have been presented in Figure 1c and d, respectively. Short-term exposure to λ-cyhalothrin caused significant decrease ($P < 0.05$) in SOD and catalase activity by 16% and 72%, respectively, when compared to control group.

The spontaneous motor activity results shows that exposure of rats to λ-cyhalothrin (7.8 and 15.6 mg/kg, *per os*) caused dose-dependent locomotor hypoactivity ($P < 0.05$) when compared to rats belonging to control group [Figure 2]. The forced locomotor activity was significantly ($P < 0.05$) altered [Figure 3] following oral administration of λ-cyhalothrin (both the dose levels i.e., 7.8 and 15.6 mg/kg) at 30, 60, and 90 min as compared to rats which received either vehicle (control) or diazepam (positive control).

In the present study, λ-cyhalothrin exhibited ($P < 0.05$) analgesic activity significantly ($P < 0.05$) in rats and it was maximum at 30, 60, and 90 min postexposure to either 7.8 or 15.6 mg/kg (*per os*) dose groups [Figure 4]. However, λ-cyhalothrin did not significantly induce any changes in onset of action, but markedly increased the duration of tonic-tonic convulsions in rats as compared to control group [Table 2].

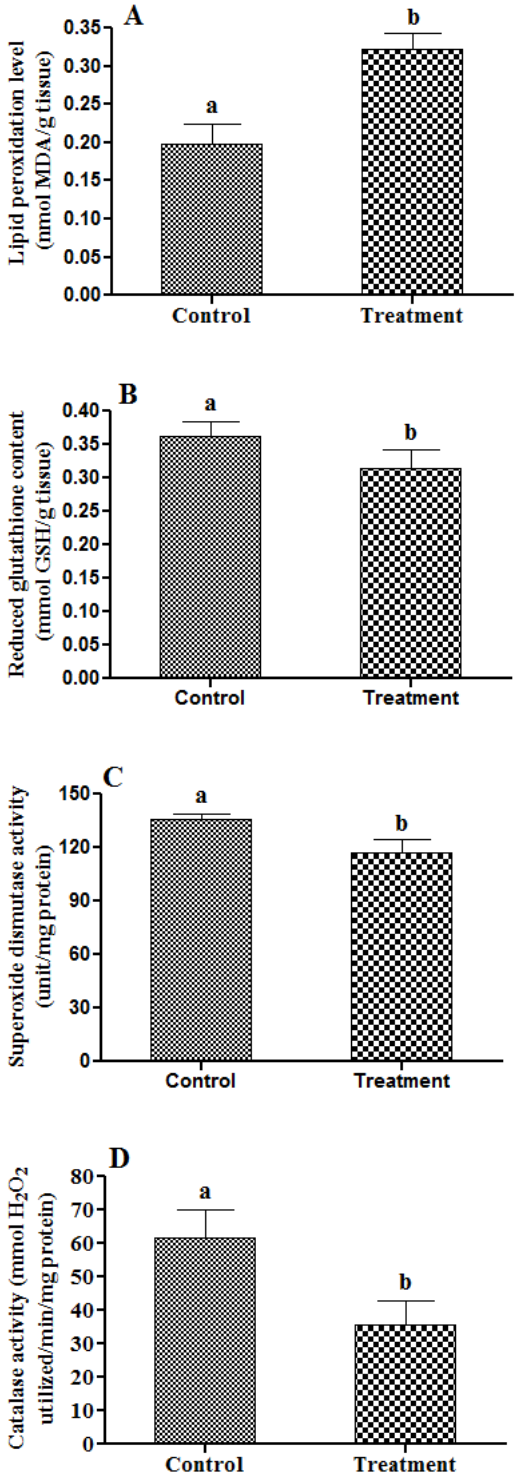


Figure 1. Effect of short-term exposure (*per os*) to λ-cyhalothrin on lipid peroxidation level **(a)**, reduced glutathione content **(b)**, superoxide dismutase **(c)** and catalase **(d)** activities in brain of rats. Each bar represents mean ± standard error ($n = 6$). Bars bearing no common superscripts vary significantly ($P < 0.05$) in "*t*"-test.

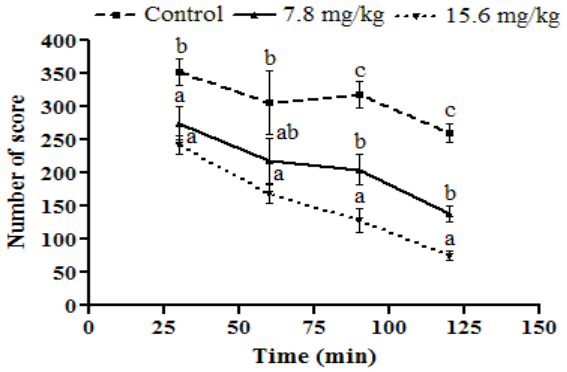


Figure 2. Effect of λ-cyhalothrin on spontaneous motor activity in rats. Each bar represents mean ± standard error ($n = 6$). Bars bearing no common superscripts vary significantly ($P < 0.05$) in Duncan's multiple comparison post-hoc test.

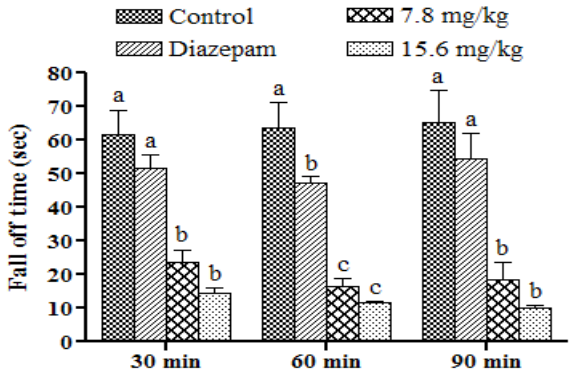


Figure 3. Effect of λ-cyhalothrin on forced locomotor activity in rats. Each bar represents mean ± standard error (n = 6). Bars bearing no common superscripts vary significantly (P < 0.05) in Duncan's multiple comparison post-hoc test.

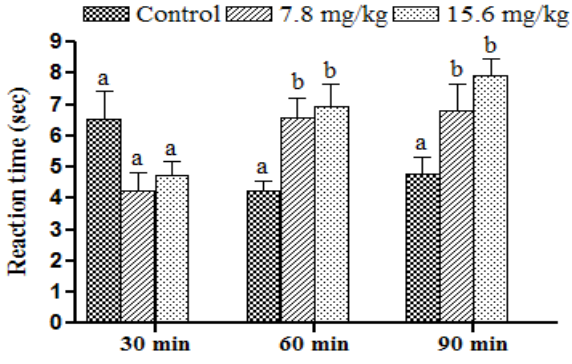


Figure 4. Effect of λ-cyhalothrin on analgesic activity in rats. Each bar represents mean ± standard error (n = 6). Bars bearing no common superscripts vary significantly (P < 0.05) in Duncan's multiple comparison post-hoc test.

Table 1. Evaluation of λ-cyhalothrin following per oral administration of at 7.8 mg/kg using modified Irwin test

Parameter	Nature of effects	Time in min		
		0–10	10–20	20–30
Ptosis	Autonomic			
Exophthalmia				
Miosis				
Mydriasis				
Defecation/diarrhea		+++		
Salivation		+++	++	+
Lacrimation				
Catalepsy	Motor			
Akinesia				
Abnormal gait (rolling)				
Abnormal gait (tip-toe)				
Motor in coordination				
Loss of balance				++
Loss of traction				
Loss of grasping				
Deceased activity: Marked	Sedation			
Deceased activity: Moderate		++	+	+
Deceased activity: Slight				
Deceased reactivity to touch				++
Decreased fear			+	+
Decreased abdominal muscle tone				+
Fore-paw treading	Stereotypy			
Head twitches		+++	+	
Stereotypies (head movements)		++		
Stereotypies (chewing)		+++	+	
Stereotypies (sniffing)		+		
Scratching		++		

Table 2. Effect of λ-cyhalothrin on strychnine-induced (2 mg/kg; i.p) convulsions in rats

Time (min)	Group I	Group II	Group III
Onset of action (min)	06.52±0.59[a]	11.15±1.09[b]	07.61±0.89[a]
Duration tonic-clonic convulsions (s)	17.00±1.86[a]	16.00±2.44[a]	21.17±1.35[a]
Number of rats died (≤30 min)	2/6	1/6	0/6

Values (mean±SE; n=6) bearing no superscripts common vary significantly (P<0.05) in Duncan multiple comparisons post-hoc test. SD= Standard deviation

15. Discussion

Apart from wide spread use of λ-cyhalothrin in agriculture, it is one of the most commonly used candidate in public and animal health protection programs by way of control of broad spectrum of insects including cockroaches, flies, mosquitoes, and ticks. In the present study, λ-cyhalothrin treated rats (7.8 mg/kg body weight) showed prominent motor, autonomic, and sedation symptoms. Similarly, Mate *et al.*,[19] reported that toxic manifestations included profuse salivation, mild fur erection, exophthalmia, abnormal gait and posture, hyperesthesia, tremors, respiratory distress, and death. However, similar clinical signs with slight variations have been observed in rats subjected to fenvalerate exposure.[20]

The brain tissue is highly susceptible to LPO because of its high rate of oxygen utilization, an abundant supply of polyunsaturated fatty acids, a deficient antioxidant defense, and a high content of transition metals such as copper and iron.[21] According to WHO,[22] pyrethroids indirectly generates various reactive oxygen species (ROS) such as superoxide radical and hydroxyl radical, and reactive nitrogen species such as peroxynitrite and nitric oxide. In the present study [Figure 1a], LPO activities of brain were significantly increased in λ-cyhalothrin intoxicated rats as compared with control rats, confirmed the susceptibility of brain tissue to oxidative stress. Therefore, oxidative damage induced by λ-cyhalothrin in the present study may be attributable to its lipophilic nature or consequence to its penetration via cell membrane and accumulation in biological membranes leading to increased production of ROS thus causing oxidative damage to its lipids and proteins. Similar observations were also made by Piner and Uner.[23]

GSH is an endogenous antioxidant and protects cells against ROS.[24] The enzymatic antioxidants, SOD, and catalase are the first line of defense against oxidative stress. GSH is the most prevalent nonprotein thiol in animal cells. Brain GSH and other thiol containing proteins play a crucial role in cellular defense against toxicity of pesticides.[25] In the current study [Figure 1b], the significant decrease of GSH content in λ-cyhalothrin treated group could be a decreased synthesis or increased utilization in the brain tissues. These results are in agreement with that of the previous studies of Agrawal and Sharma,[3] Hussien *et al.*,[26] and Skrivan *et al.*[27] Recently, it has been reported that depletion of GSH levels with concomitant increase in LPO is one of the mechanisms of λ-cyhalothrin toxicity.[4]

The detoxification of ROS in brain involves the co-operative action of all intracellular antioxidant enzymes. SOD is the first line of antioxidant enzymatic defense that catalyzes the conversion of superoxide radicals to less toxic H_2O_2 and catalase metabolizes H_2O_2 to water. When this mechanism is saturated, the second line of antioxidant enzymatic defense is mainly GSH peroxidase.[28] In the present study, λ-cyhalothrin decreased the activities of SOD and catalase [Figure 1c and d] in the brain indicating suppression of the antioxidative defense mechanisms or may be attributed to their mutual functioning for eliminating ROS. The current data are in accordance with previous results, which reported a decreased activity of antioxidant enzymes in rats exposed to pyrethroids.[5,26,29]

Spontaneous motor activity is one of the most extensively studied behaviors in rats and mice. Motor activity reflects functional output of the CNS and it is the behavior that is relevant to survival. Our data are consistent with earlier observations that motor function is a part of behavior affected by all pyrethroids independent of the route of administration or species exposed, and it is the most extensively characterized neurobehavioral endpoint of pyrethroid intoxication.[30] The vast majority of pyrethroids of type I and II produce a dose-related decrease of general motor activity in mammals.[30] Our present study shown that exposure to λ-cyhalothrin significantly reduced locomotor activity in rats, which is in tune with the study of Nieradko-Iwanicka and Borzecki.[31] The decreased spontaneous motor activity and forced locomotor activity in λ-cyhalothrin treated rats suggested that there may be depression of CNS resulting in motor in-coordination due to the distinct inhibitory action on motor performance involving various central neurotransmitters. In the present study, the rotarod test showed that "fall off" time in rats decreased at the both dose (7.8 and 15.6 mg/kg) at all times and this decrease in "fall off" time was determined to be statistically different from the control as well as diazepam-treated group. In a similar study, using grip strength test has been reported that pyrethroids weaken the neuromuscular response in rats.[30] Similar results were also observed in rats, which received cypermethrin at different dose levels.[32,33] Further, λ-cyhalothrin showed decreased motor activity in comparative acute toxicity study with a bench mark threshold dose of 0.5 mg/kg (*per os*) in rats when data was subjected to a nonlinear exponential threshold model.[34]

The predominant molecular targets of pyrethroids include neuronal voltage-sensitive sodium channels (VSSCs).[35] Although voltage-sensitive Ca^{+2} channel (VSCC) isoforms[36] and voltage-sensitive Cl^- channels[37] are involved in toxicity of type II pyrethroid, their exact role is least characterized. Ray and Fry[38] opined that the concentrations required to pharmacologically alter the function of the VSSC, VSCC, and Cl^- channel types are very similar. It has been reported that displacement of $GABA_A$ receptor ligands by type II pyrethroids occurs at much higher concentrations than those required to produce effects on VSSC-mediated Na^+ currents, and also blockade of Cl^- influx occurs at even higher concentrations.[38] Although we did not measure the tissue (CNS) concentrations of either cyhalothrin or the levels of neurotransmitters in CNS, it is imperative that the impairment in neuropharmacological activity after cyhalothrin treatment in the current study may be due to the oxidative stress and/or disturbances in integrated central neuronal circuit.

Interestingly, in the present study, λ-cyhalothrin significantly enhanced the analgesic activity in rats. It has been reported that Icon®, a type II synthetic pyrethroid insecticide based on active ingredient λ-cyhalothrin (10% w/w), also exerted antinociceptive action in pregnant rats in dose-dependent manner following *per os* exposure.[39] Further, the reaction time was highest during last stage of pregnancy (day 15–21) when rats treated with λ-cyhalothrin (12.5 mg/kg/day × 7 days, *per os*) were subjected to assay for analgesic using hot plate technique. Cyhalothrin being a type II pyrethroid can act on sodium channels in neurolemma to disrupt proper functioning of neurons in the CNS to induce antinociception.[40] On the other hand, the raise in nociceptive threshold observed in the present study may be due to direct effect of pyrethroid on GAB_A receptors and spontaneous release of GAB_A,[41] which triggers Cl^- entry into the postsynaptic neuron leading to hyperpolarization of the postsynaptic neuron and decrease in neuronal excitability. Supraspinally organized responses and sensitive to phasic and noninflammatory pains can be determined by hot plat test.[42] Thus, the analgesic effect observed in the current study is predominantly due to central mechanisms. Further, noticeable signs of sedation and stereotypy after λ-cyhalothrin ($1/10^{th}$ LD_{50} treatment group) also indicated CNS-mediated analgesic activity.

To conclude, short-term exposure to λ-cyhalothrin causes oxidative stress by altering the redox homeostasis in brain associated with reduction in both the enzymatic and nonenzymatic antioxidative defense mechanisms. Further, a relation between the effects of λ-cyhalothrin on neuropharmacological paradigms following single sub-lethal exposure in Wistar rats is hypothesized.

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