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Beta-cyfluthrin induced neurobehavioral impairments in adult rats

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ABSTRACT

Beta-cyfluthrin (CYF) is a commonly used synthetic pyrethroid having both agricultural and domestic applications. The present study aimed to evaluate the neurobehavioural effects of beta-cyfluthrin in adult rats administered at doses 25 mg/kg body weight/day and 12.5 mg/kg body weight/day for a period of 30 days. Motor coordination and spatial memory were found to be impaired by beta-cyfluthrin. Levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), epinephrine (EPN), and serotonin (5-HT) decreased in frontal cortex, corpus striatum and hippocampus of treated rats. At the same time, significantly elevated levels of homovanillic acid (HVA) and nor-epinephrine (NE) were measured. Beta-cyfluthrin inhibited the activity of acetylcholinesterase (AChE) in all the regions of the brain. Hippocampal choline acetyltransferase (ChAT) expression was reduced 3.1 and 4.7 fold by the two doses respectively. Impairment of the antioxidant defense system, evident by decrease in the levels of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) was seen in the treated rats. The neurochemical alterations manifested were more pronounced in the high dose group as the effects persisted even after withdrawal of exposure.

1. Introduction

Pyrethroids are presently the most widely used class of pesticides, being used in agriculture, forest, textile industry and public health programs worldwide [1]. These are the structural derivatives of naturally occurring pyrethrins, which are present in pyrethrum, an extract from the flower *Chrysanthemum cinerarifolium* [2,3]. The basic pyrethroid structure was modified to increase its insecticidal potency and photostability but this also resulted in changes in pyrethroid activity in non-target species [4–7]. The addition of α-cyano group to the alcohol moiety proved to be a milestone in the development of synthetic pyrethrin analogs as this modification resulted in enhancement of their insecticidal activity [8,9]. Acute toxic studies in rodents report an increased potency of approximately one order of magnitude due to this chemical group [6,10].

Despite being considered relatively safe for humans; epidemiological data, clinical reports and laboratory studies indicate that pyrethroid exposure leads to neurotoxic and immunotoxic effects in humans and animals [11,12]. Exposure to these compounds has also been linked to acute reproductive effects and developmental deficiency [13,14]. Behavioral deficits associated with pyrethroid exposure including impairment of motor activity, grip strength, learning ability have been well documented [15–18].

Beta-cyfluthrin, the refined form of synthetic pyrethroid cyfluthrin, is currently being used in many formulations around the world. It belongs to the Type II class of synthetic pyrethroids and acts as a contact and stomach poison. It is used to control a wide variety of both indoor and outdoor pests including roaches, silverfishes, fleas, spiders, ants, crickets, houseflies, ticks, mosquitoes, wasps and more. In addition to its household applications, cyfluthrin is also widely used in various public health programmes [19].

The neurotoxic effects of cyfluthrin and other pyrethroids are primarily mediated through their interaction with sodium channels, leading to depolarization and hyperexcitation of the nervous system [20–22]. This group of compounds has also been shown to act on isoforms of voltage sensitive calcium channels [23], thereby contributing to the release of neurotransmitters and hence leading to pyrethroid induced toxicity [24]. Biogenic amines are a group of neurotransmitters viz. dopamine, norepinephrine and serotonin which modulate a variety of cognitive and behavioral functions. Alteration in their levels leads to neurobehavioural changes [25]. The cholinergic system is another potential target for the action of pyrethroids [26,27]. Induction of oxidative stress is an important

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mechanism involved in pesticide induced toxicities; damage to DNA, proteins and membrane lipids being the major endpoints [28,29]. SOD, CAT and GPx are the antioxidant enzymes which can play a vital role in protecting these molecules from free radicals generated oxidative stress [30].

The increased use of pyrethroids has made human exposure almost inevitable. Parkinson's disease (PD) is a neurodegenerative disorder affecting millions of people globally [31]. Among the various environmental risk factors involved in the etiology of PD, strong association exists between pesticide exposure and occurrence of PD [32]. It involves neurodegeneration of dopaminergic neurons, mainly in the substantia nigra, but also in other brain areas such as the cortex and hippocampus [33,34]. PD is primarily manifested by effects on motor function, including abnormalities of movement, gait and balance [35]. Several studies report the effects of pyrethroids on dopaminergic nerve pathways [36,17] which may be a factor involved in environmentally induced PD.

The present study was therefore undertaken to investigate the toxic effects of beta-cyfluthrin on motor coordination, spatial learning and changes in the levels of biogenic amines following exposure of adult rats for 30 days. Further, to assess whether these changes were transient or persistent, the behavioral and neurochemical studies were again conducted 15 days after withdrawal of beta-cyfluthrin exposure.

2. Materials and methods

2.1. Animals and treatment

Adult male and female Wistar rats weighing around 120 ± 5 g were procured from the Indian Veterinary Research Institute (IVRI), Bareilly and a colony was maintained. Rats were housed in an air cooled vivarium at 25 ± 2 °C with a 12-h light/dark cycle under standard hygienic conditions. Animals had a free access to pellet diet procured from Ashirwad Industries, Chandigarh and tap water. The experimental protocol was approved by the Departmental Ethical Committee (1678/GO/a/12/CPCSEA).

Male rats aged 60 days and average weight 150 ± 5 g were selected for the study. Rats (n = 66) were divided into three groups. Beta-cyfluthrin (purity >99%) procured from Sigma–Aldrich (St. Louis, MO, USA), dissolved in corn oil was administered orally at doses of 25 mg/kg body weight (1/15 of LD_{50}) or 12.5 mg/kg body weight (1/30 of LD_{50}) in two groups of rats daily for a period of 30 days. The LD_{50} of beta-cyfluthrin in rats is reported to be 380 mg/kg body weight [37,38]. The third group was administered corn oil similarly and it served as control.

After 30 days of treatment, randomly selected animals (n = 5) from each group were subjected to behavioral studies, a set of animals (n = 17) from each group was sacrificed for neurochemical observations. Brains were immediately removed, washed in ice cold saline and dissected into frontal cortex, corpus striatum and hippocampus [39] and processed for biochemical assays. To explore whether the neurobehavioural alterations produced by beta-cyfluthrin were transient or persistent a set of animals from each treatment group were left as such for 15 days. Behavioral and neurochemical endpoints were then observed on these animals.

3. Behavioral studies

3.1. Rota-rod performance

Motor coordination and balance of animals was studied using a rota-rod (IMCORP, Ambala, India) following the procedure described by Kumar et al. [40] with slight modification. A set of animals from each treatment group was trained to stay on the rotating rod, until it achieved a criterion of staying on the rod for 60s. The final observations were taken by placing the rats on the rotating rod (25 rpm) with 180s as the cut-off time. The time of fall from the rotating rod was recorded as a measure of motor coordination, scoring was carried out by a person blind to the treatment condition. Each rat was subjected to three consecutive trials after a gap of 5 min.

3.2. Morris water maze

The Morris water maze (MWM) test was used to assess the spatial learning in rats following the procedure described by Chen et al. [41] with slight modification. The maze consists of a large circular tank, (150 cm diameter, 50 cm height) filled with water (28 ± 2 °C) and placed in the center of a large room with extra-maze cues. The water tank was divided into four equal quadrants: south–west (SW), north–west (NW), north–east (NE) and south–east (SE). A square platform (10 cm^2) was submerged 2 cm below the water surface, located in the center of one of the quadrant. The training procedure was divided into two phases (phase-1 and phase-2).

Phase-1 (place navigation): Rats were given four training trials each day for 4 consecutive days. The trials began on day 26th of treatment and for the withdrawal part of the study the training session began on 11th day following withdrawal of exposure. For each training trial, the rats were placed in the water facing the pool wall at one of four positions (at the north, south, east or west pole) in a different order each day, and were allowed to swim until they reached the platform. The time required to reach the platform was recorded for up to 120 s (escape latency). The rats were kept on the platform for 10 s before removal.

Phase-2 (spatial probe test): After 4 days of training, the platform was removed from the tank and a 120 s spatial memory retention test was conducted. The animal uses the extra-maze cues as reference to navigate and reach the platform. When the platform is removed from the tank, the animal utilizes the spatial information acquired during the training task and traverses the platform site. Spatial memory of animals is manifested by the time spent in the quadrant where the platform was placed.

4. Neurochemical studies

4.1. Biogenic amines and metabolites in brain regions

Estimation of dopamine (DA), nor-epinephrine (NE), epinephrine (EPN), serotonin (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in frontal cortex, corpus striatum and hippocampus was carried out by reversed phase high performance liquid chromatography (HPLC) with electrochemical detector following the method of Kim et al. [42] with minor modifications. The HPLC system (Waters, Melford, USA) consisted of a high-pressure isocratic pump (515 HPLC Pump), a sample injector valve, C-18 reverse phase column (250 mm × 4 mm, particle size 5 μm) and electrochemical detector (464 Pulsed electrochemical detector). Briefly, brain regions were homogenized in 0.1 M perchloric acid containing 3,4-dihydroxybenzylamine, an internal standard at final concentration of 25 ng/ml followed by centrifugation at 36,000× *g* for 10 min. The supernatant obtained was then filtered through 0.25 mm nylon filters (Millipore, USA) and used for the determination of levels of biogenic amines. 20 μl of sample volume was injected in injector port. The mobile phase (pH 4.2) containing sodium dihydrogen phosphate (0.15 M), ethylenediaminetetra acetic acid (0.25 mM), sodium octyl sulfate (1.75 mM) and 4% methanol at a flow rate of 1.5 ml/min was used to separate peaks in the samples. Amperometric electrochemical