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Effects of cypermethrin exposure on learning and memory functions and anxiety-like behavior in rats

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Abstract

Background Cypermethrin (CYP), a synthetic pyrethroid widely used to control plant pests, has been associated with various diseases in humans exposed to pesticides, either directly or indirectly. This study aimed to examine the effects of CYP on learning and memory functions, as well as anxiety-like behavior.

Methods Forty male Wistar rats (8 weeks old) were randomly assigned to 4 groups: The first group served as the control, while the other three groups received different doses of CYP (5, 20, and 80 mg/kg) via gavage once daily for one month. Passive avoidance learning (PAL) and memory were assessed using the shuttle box test, cognitive memory was evaluated using the novel object recognition (NOR) test, and spatial memory was measured with the Morris water maze (MWM) test. The elevated plus-maze (EPM) and open field tests were used to assess locomotor activity and anxiety levels.

Results In the PAL test, significant differences were observed in the time spent in the dark compartment (TDC) and step-through latency in the retention trial (STLr) in rats receiving 80 mg/kg of CYP. MWM results indicated memory impairment in rats treated with 20 and 80 mg/kg of CYP. Additionally, rats treated with the highest dose of CYP (80 mg/kg) showed a reduction in the number of entries into the open arms of the EPM compared to the control group.

Conclusion This study demonstrates that CYP negatively affects learning and memory retention. Further research is needed to explore the precise mechanisms by which this toxin impacts cognitive functions.

Keywords Cypermethrin, Locomotor activity, Passive avoidance learning, Spatial learning and memory, Elevated plus-maze

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Introduction

Pyrethroids (PYRs) are a class of synthetic insecticides derived from Chrysanthemum cinerariifolium [1]. They are widely used to control domestic insects, agricultural pests, and malaria vectors through indoor spraying, spatial spraying, and mosquito nets impregnated with pesticides [2, 3]. Pyrethroids are axonic excitotoxins that have harmful effects on ion channels in the nervous system, disrupting the entry and passage of ions across cell membranes [4–6]. The most significant pathological mechanism of PYRs is their ability to alter sodium channel activity, leading to neurotoxicity [7].

Key pyrethroids include allethrin, cypermethrin (CYP), permethrin, fenvalerate, deltamethrin, and λ-cyhalothrin [1]. These compounds typically feature cyclopropane carboxylic acid or similar structures attached to aromatic alcohols via a central ester (or ether) bond. Modifying the structure of pyrethroids can enhance their insecticidal effectiveness, but this may also lead to unintended consequences for non-target species [8]. Among synthetic pyrethroids, CYP is a highly effective, non-systemic insecticide that works through contact and gastrointestinal effects and is commonly used worldwide for pest control in agriculture and public health [9]. Oral or intravenous exposure to these toxins can cause symptoms such as salivation, itching, increased muscle tone, balance disorders, tremors, seizures, apnea, and, in severe cases, death [10].

One example of a pyrethroid structural modification is the addition of an α-cyano group, as seen in CYP, which results in a type II pyrethroid [11]. Cypermethrin, or [cyano-(3-phenoxyphenyl) methyl]3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate, is a synthetic pyrethroid with potent insecticidal properties [12]. CYP toxicity can cause various disorders in humans, whether through direct or indirect contact with the pesticide. Children and farmers are particularly vulnerable [13]. Mammals are generally more resistant to CYP and other pyrethroids due to a high metabolic rate, elevated intracellular temperature, and low sensitivity in target sites [14]. Type II pyrethroid poisoning, such as from CYP, can cause paralysis, dizziness, vomiting, and muscle fasciculation when absorbed through the skin [15], while oral poisoning leads to salivation, pulmonary edema, seizures, and coma [16].

Extensive exposure to pyrethroids through unclear mechanisms has been linked to neurological symptoms, including cognitive impairment [17, 18]. There are also reports indicating that type II pyrethroids like CYP can impair memory [4, 19]. Learning and memory are essential behavioral traits that reflect an organism's ability to adapt based on experience [20, 21]. Learning involves changes in behavior through practice, while memory sustains these behavioral changes over time [22]. Numerous studies have shown that CYP, as an emerging neurotoxin, can cause psychological and behavioral changes, with long-term complications [23]. CYP disrupts nerve function by inhibiting the deactivation of sodium channels, which stabilizes the open configuration of the channel. This prolonged axonal depolarization leads to conduction blocks and neuromuscular transmission failure, resulting in flaccid paralysis [24–29]. The present study aims to evaluate the effects of CYP on learning and memory functions, specifically cognitive memory, spatial learning and memory, anxiety-like behavior, and passive avoidance learning (PAL) in adult male rats. Our primary goal is to provide accurate data on the harmful impacts of CYP to limit its widespread use, ultimately contributing to a healthier and more sustainable environment for all.

Materials and methods

Animals

This research was conducted on 40 adults male Wistar rats (8 weeks old) weighing 250 ± 20 g, which were purchased from the animal lab of Hamadan University of Medical Sciences. The rats were accommodated in a ventilated room at 22 ± 2 °C under a 12-h light/dark cycle and humidity of 50-55%. 2 or 3 rats were kept in cages, and food and water were easily accessible. Rats were housed in standard cages measuring 30 cm x 20 cm x 15 cm (L x W x H). All animal experiments were approved by the Veterinary Ethics Committee of Hamadan University of Medical Sciences and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85 – 23, revised 1985). The rats were randomly assigned to four groups: a control group receiving corn oil and three experimental groups administered CYP at doses of 5, 20, and 80 mg/kg, dissolved in 0.2 ml of corn oil. CYP and corn oil were administered orally via gavage once a day for one month (Fig. 1). After the administration of the agents, the animals were tested and acclimated to the laboratory conditions one hour before the start of each test.

Chemicals

Cypermethrin (PESTANAL®) and corn oil (maize oil) were prepared from Sigma Aldrich Company (St. Louis, USA). CYP was dissolved in 0.2 ml Corn oil [30].

Open field test (locomotor activity)

The standard open field test (OFT) is used to measure behavioral reactions, including locomotor and exploratory behaviors [31, 32]. The apparatus consists of a square Plexiglas box (76 × 76 cm) with opaque walls and a floor divided into 25 identical squares, with a height of 42 cm. A white curtain enclosed the apparatus to eliminate extra-maze cues. Rats were placed in the central square to monitor their activities. The apparatus was

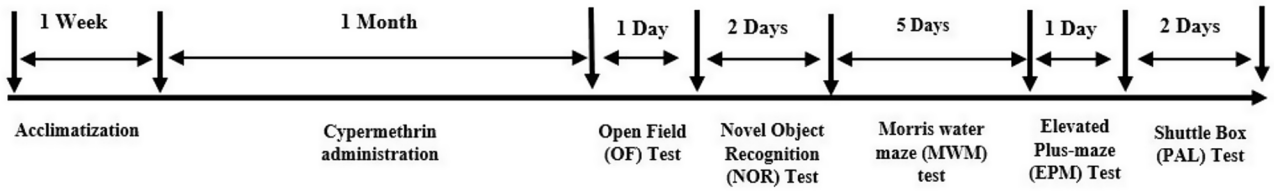

Fig. 1 The experimental timeline. After one week of acclimatization, animals received different doses (5, 20, and 80 mg/kg) of cypermethrin (CYP) by gavage daily for one month. The novel object recognition (NOR) and open field (OF) tests were then conducted. To MWM and passive avoidance learning (PAL) tests measured spatial (retention and acquisition) and aversive (retention and acquisition) learning and memory after the training trials, respectively. The elevated plus-maze (EPM) test assessed the animal's locomotor activity (in addition to the OF test) and level of anxiety

cleaned with tap water followed by 70% ethanol before each subject's trial and after the last run. On the testing day, all rats were transferred to the experimental room and allowed to acclimate for 60 min. Testing began immediately after placing each rat in the central region of the apparatus. The illumination level was set at 325 lx during the test, and the apparatus was located in a partially soundproof room. To assess the impact of CYP on locomotion, the total distance traveled (cm) during 10 min was recorded using the Maze Router homemade software.

Assessment of spatial memory via Morris water maze (MWM) test

Learning and spatial memory were evaluated via an apparatus called MWM specifically designed for this task [33]. A black cylindrical pool (diameter: 180 cm and height: 60 cm) filled to a depth of 25 cm with tap water (24 ± 1 °C), was used to conduct this experiment. A fixed round platform (10-cm2) was located in the center of the southwest quadrant, 1 cm beneath the water level. The experiment consisted of training tests for 4 days and a probe test 24 h following the final training session [34].

In each training test, the animal was first put in the water in 1 of the 3 quadrants with no platform and could swim freely for 90 s. If an animal succeeded in finding the platform before the end of the 90s, it was removed from the platform after 10s, dried with a thick high-absorbing towel, and put in its cage. However, if the platform was not found, the subject was gently positioned on the platform and stayed there for 30 s, after which it was picked up, dried, and put in its cage. On every training day, each animal was trained 4 times and rested 30 min between trials. The probe test was conducted to examine the spatial memory of the subjects. On each trial, the time between entrance into the water and escape toward the platform (escape latency) was calculated. 2 blocks of 4 trials were used to train the animals for the spatial learning task with an interval of 5 min. The rats were subjected to 4 consecutive daily training trials at the same time each day (10:00–12:00). The trials were recorded by smart video tracking software. The escape latency, the time elapsed in the target quadrant, mean speed, and distance moved were applied to assess the animals' spatial learning and memory [33, 35, 36].

Passive avoidance learning test

The shuttle box was used for conducting a passive avoidance learning test [36–39]. It was a cuboid container (25 × 25 × 50 cm), divided into dark and light compartments (25 × 25 × 25 cm) isolated by a one-way guillotine door (10 × 10 cm). The dark compartment's floor was a steel-rod grid (2.5 mm in diameter and 1 cm apart), which is connected to an electric shock generator (50-Hz, 1-mA), and a 100-watt lamp was installed 40 cm above the floor to illuminate the light compartment [40]. The rats were habituated to the apparatus in 2 trials with 30 min intervals. According to the rats' natural preference for the dark places, for each trial, the rat was located in the apparatus's light chamber, in front of the door, and after 10 s, the guillotine door was lifted. Following entrance to the dark chamber, the door was raised for 10 min, and then the rat was removed from the dark chamber and transferred to its cage. Then, after habituation trials with 30 min intervals, in the initial acquisition trial, after the entrance of the animal to the dark chamber from the light compartment spontaneously and placing 4 paws in the dark chamber from the light chamber, the entrance latency to the dark compartment (STLa) was measured. The guillotine door moved down, and the rat's foot received an electric shock (0.3 mA, 50-Hz square wave, for 3 s). The animal was transferred to its home cage after 30 s. After 2 min, the training procedure was done again. When the animal stayed in the light compartment for 120 successive seconds, the training phase was ended. The number of entrances into the dark compartment was noted.

A retention test was performed 24 h following the training to assess the trained animals using the same process as that in the training session but without any shock in the dark chamber and the animals remained in the dark chamber up to 600 s. We recorded the entrance latency to the dark chamber or step-through latency in the retention trial (STLr) and the time elapsed in the dark compartment (TDC) for 10 min [41, 42].

Elevated plus-maze (EPM) test

This test was used to assess the rat's anxiety-related behavior and was conducted in a wooden apparatus shaped like a plus sign [43, 44]. The apparatus is 50 cm in height and consists of two enclosed arms (50 × 10 × 40 cm) and two open arms (50 × 10 cm). A 10 × 10 cm central square is located at the junction of the opposite arms, where the different arms meet. Each rat was placed in the center of the apparatus, facing an open arm, and allowed to explore the device for 10 min. During this time, all entries into the enclosed or open arms were recorded. Further assessments included the time spent in the open and enclosed arms, as well as the total distance traveled during the 10-minute period. A rat was considered to have entered an arm when all four paws were placed within the arm. Behavioral measures included the number of entries into the open and closed arms, the time spent in each arm type, and the total distance traveled (TDC) during the test. An entry was defined as all four paws of the rat entering an arm. A lower number of open arm entries and less time spent in open arms were interpreted as anxiety-like behaviors [45, 46]. To control for potential confounding effects of reduced locomotor activity, TDC was measured as an index of general motor activity. This ensured that any observed anxiety-like behaviors, such as increased time in closed arms, were not influenced by impaired movement.

Novel object recognition (NOR) test

This test was designed to assess memory in rodents [47–49]. The subject was exposed to one or more familiar objects and one novel object. The Novel Object Recognition (NOR) test consists of three phases: habituation, training, and retention. In the first phase, the subject was allowed to explore an empty arena (a cubic container measuring approximately 40 cm in length, width, and height) for 5 min. After this, the subject was removed from the arena and returned to its holding cage. In the training phase, the subject was placed in the container for 10 min with two familiar objects (rectangular hard items positioned 10 cm from the wall of the box). To avoid forcing the subject to explore the objects, it was positioned facing the midsection of the wall opposite the objects. After 30 min, in the retention test phase, each animal was placed back into the arena for 5 min, during which one of the familiar objects was replaced with a novel object. The expected behavior was for the subject to explore the novel object more than the familiar one. If the subject failed to show more interest in the novel object, memory impairment was concluded. Throughout each phase, a video tracking system recorded the animal's behavior, and the time spent exploring each object was measured. The objects used were similar in material and size, but differed in shape. To eliminate olfactory cues, both the items and the arena were cleaned with 70% alcohol and air-dried thoroughly between trials. A camera recorded the touching and sniffing of the objects by the animal's nose, which was used to measure exploration time. Finally, the discrimination index (DI) was calculated as the ratio of the total time spent exploring the new object to the total time spent exploring both objects, multiplied by 100 [50, 51].

Method of euthanasia

Rats were euthanized at the conclusion of the experiment using intraperitoneal (IP) injections of sodium thiopental. This method of euthanasia is classified as chemical euthanasia, involving an overdose of an anesthetic agent. The recommended dose of sodium thiopental for IP euthanasia in rats typically ranges from 100 to 150 mg/kg. Following the IP injection, the animal enters a deep, irreversible state of anesthesia, leading to respiratory and cardiac arrest. Common indicators of death include the absence of a heartbeat, lack of spontaneous breathing, and unresponsiveness to external stimuli.

The decision to euthanize the animals was made to minimize potential distress following prolonged behavioral assessments, which can be stressful and affect animal welfare. Since no tissue samples were collected for histological or molecular analysis in this study, euthanasia was performed to avoid any unnecessary prolonged suffering.

Data analysis

SPSS 16 was used for data analysis at a significance level of 0.05. One-way analysis of variance (ANOVA) was applied for multiple comparisons, followed by the Tukey's test for post-hoc analysis. Data are presented as mean ± SEM.

Results

Locomotor activity (open-field)

The OFT results demonstrated that CYP administration at 80 mg/kg significantly decreased total distance traveled compared to the control group ($P < 0.001$), indicating reduced locomotor activity (Fig. 2). This measure served as the primary indicator of motor function in this test. Lower doses of CYP (5 mg/kg and 20 mg/kg) did not show significant changes in total distance traveled relative to controls.

Effect of cypermethrin on spatial memory (MWM task)

No significant difference was detected in escape time between the four groups (data for day four: control: 12.49 ± 1.47; CYP (5 mg/kg): 12.4 ± 0.9; CYP (20 mg/kg): 14.1 ± 1.3; CYP (80 mg/kg): 12.4 ± 1.01) (Fig. 3.A). Therefore, rats in all groups were able to learn the task.

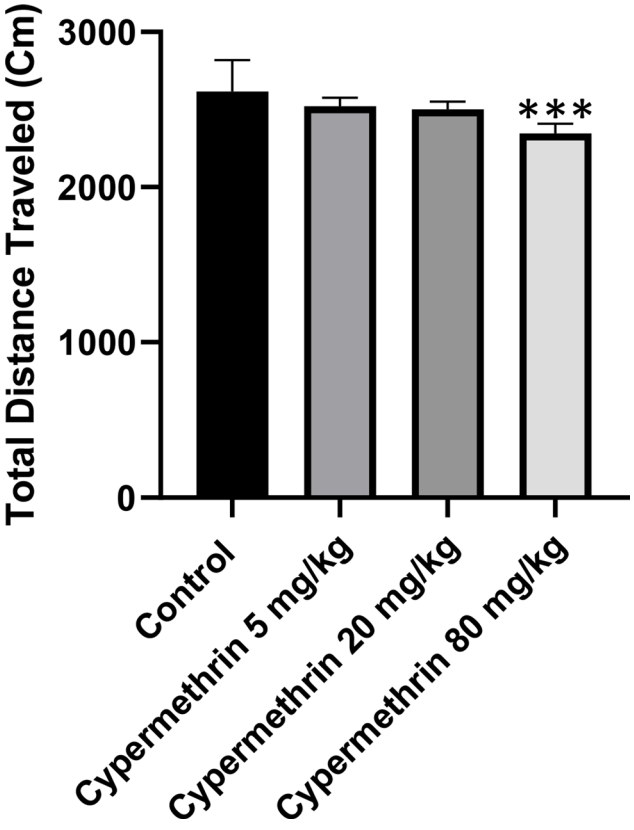

Fig. 2 Cypermethrin (5, 20, and 80 mg/kg) effects on locomotor activity in the open field test. The mean and standard deviation for the total distance traveled of at least two independent experiments are presented; $n = 10$ rats for each group. $^{***}P \leq 0.001$ than the control group. Significance refers to one-way ANOVA

Significant differences were observed between the dosed and control groups, as shown in Fig. 3.B ($P \leq 0.0001$). Tukey's test revealed a significant decrease in distance traveled on days three ($P \leq 0.001$) and four ($P \leq 0.001$) in the CYP (20 and 80 mg/kg) groups compared to the control group. Figure 3.C shows a significant difference in the time spent by rats in the target quadrant between the dosed groups. One-way ANOVA results indicated that rats treated with CYP (80 and 20 mg/kg) spent significantly less time in the target quadrant compared to controls ($P \leq 0.0001$ and $P \leq 0.01$, respectively). Additionally, one-way ANOVA results showed a significant decrease in swimming speed for the CYP (80 mg/kg) group compared to controls (Fig. 3.D).

Effect of cypermethrin on PAL test

One-way ANOVA results indicated no significant differences between experimental groups regarding STLa in the first acquisition trial (prior to administering an electrical shock) (control: 7 ± 1.03; CYP (5 mg/kg): 7.6 ± 0.7; CYP (20 mg/kg): 7.9 ± 0.53; CYP (80 mg/kg): 7.37 ± 0.9) (Fig. 4.A). However, a significant difference was observed in STLr during the retention test (24 h after training) (Fig. 4.B). Specifically, STLr in the groups receiving CYP at doses of 20 mg/kg (258.7 ± 25.2; $P = 0.015$) and 80 mg/kg (138.75 ± 14.3; $P \leq 0.001$) was significantly lower compared to the control group (270.4 ± 10.5). Additionally, significant differences were found in TDC across the groups (Fig. 4.C). There was a significant increase in TDC in the groups receiving CYP at doses of 20 mg/kg (238.4 ± 9.1; $P = 0.005$) and 80 mg/kg (276.14 ± 13.7; $P < 0.0001$) compared to the control group (222.17 ± 10.5).

Effect of cypermethrin in EPM test performance

To account for the potential influence of general motor activity on anxiety-related behaviors, we measured total distance traveled (TDC) as an index of locomotion. The one-way ANOVA results for TDC (Fig. 5.A) showed that rats exposed to CYP (20 and 80 mg/kg) exhibited a significant reduction in TDC compared to the control group. This reduction suggests that the observed anxiety-like behaviors (i.e., reduced time spent in the open arms and fewer open-arm entries) may have been partially influenced by decreased locomotion at higher CYP doses. However, the decrease in time spent in the open arms (Fig. 5.C) and the number of open-arm entries (Fig. 5.B) remained significant even after accounting for TDC, indicating that the anxiety-related behaviors were not solely due to impaired motor activity. No significant differences in the number of entries into the closed arms were observed across groups (Fig. 5.D), further supporting the notion that the reduced open-arm exploration was due to anxiety rather than a general decrease in activity.

Effect of cypermethrin in NOR test

One-way ANOVA results declared no significant difference in the discrimination index between the experimental groups ($P > 0.05$) (Fig. 6).

Discussion

We investigated the effect of CYP on learning and memory as key aspects of cognitive function. Our findings from the NOR, MWM, and PAL tests demonstrated that CYP impaired both memory and learning. Specifically, CYP disrupted the learning process and memory recall. The results of the EPM, MWM, and PAL tests showed that exposure to high doses of CYP (20 and 80 mg/kg), compared to the low dose (5 mg/kg), caused significant differences when compared to the control group. Although the present study did not measure biochemical parameters, existing literature suggests that different pathways such as oxidative stress or inflammation could potentially explain the observed behavioral effects. These associations remain speculative and warrant further investigation in future studies.

Spatial learning and memory are commonly used indicators for assessing cognition in animal models. The role

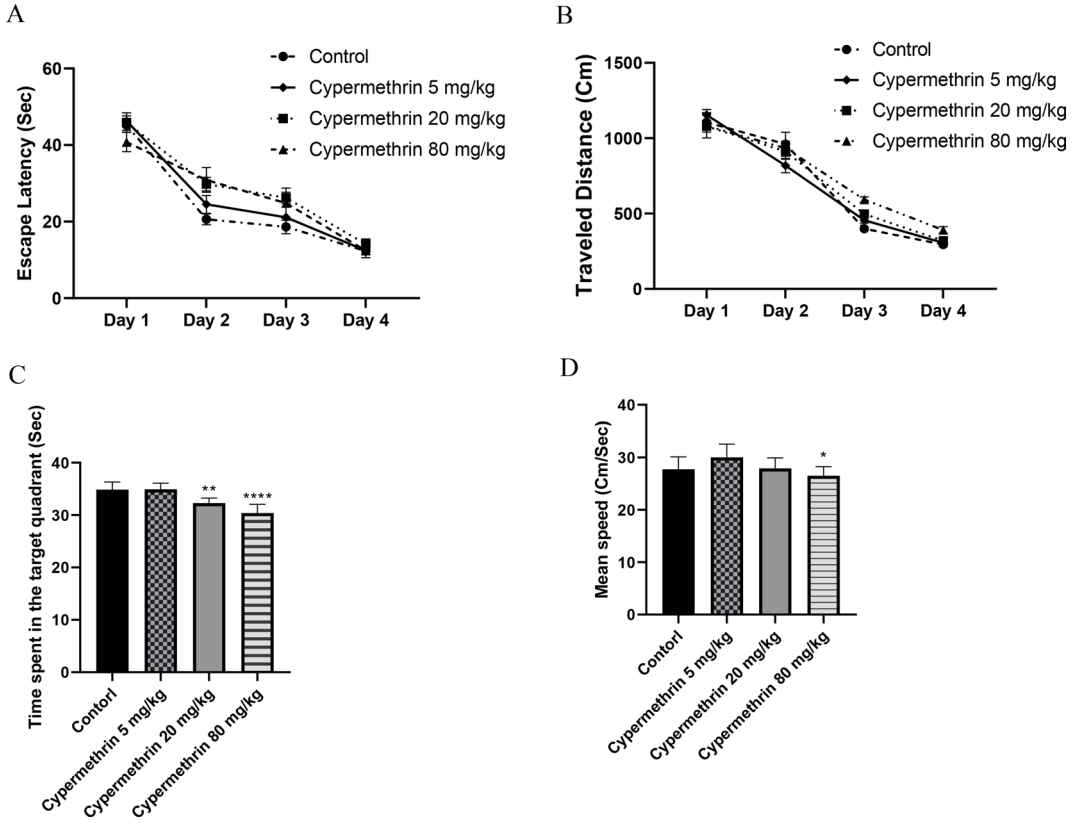

Fig. 3 Escape latency (**A**), distance traveled (**B**), time elapsed in the target quadrant (**C**), and mean swimming speed (**D**) in the Morris water maze test. Data are reported as mean ± SD for at least two independent experiments; $n = 10$ rats for each group. *$P \leq 0.05$, **$P \leq 0.01$, and ****$P \leq 0.0001$ compared to the control group. Significance refers to two-way repeated-measures ANOVA followed by the Tukey post hoc test

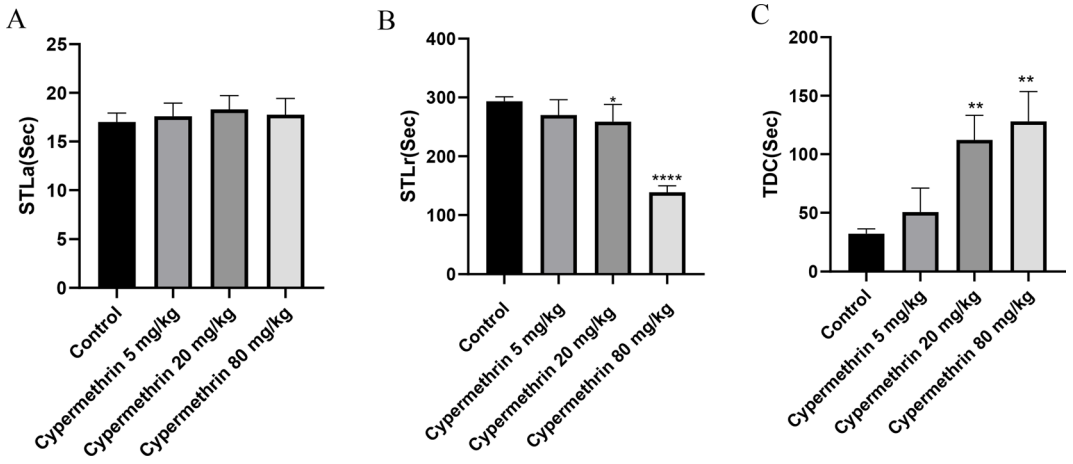

Fig. 4 Step-through latency in the acquisition trial (STLa) (**A**) and retention trial (STLr) (**B**), and time elapsed in the dark compartment (TDC) (**C**) of the passive avoidance learning test. Data are shown as mean ± SD for at least two independent experiments; $n = 10$ rats for each group. *$P \leq 0.05$, **$P \leq 0.01$, and ****$P \leq 0.0001$ compared to the control groups. Significance refers to one-way ANOVA

of the hippocampus in spatial learning and memory has been well-documented [52]. A study evaluated the effects of CYP administration on both embryonic and adult neurogenesis, showing that exposure to CYP not only reduced the pool of neural stem cells but also disrupted the neuron-astrocyte ratio, leading to neurodegeneration in the hippocampus and subsequent cognitive dysfunctions in rats [53]. The impact of CYP and deltamethrin on cerebral cortex development, as well as their potential effects on cell proliferation and differentiation, has also been studied in mice. The results indicated that prenatal exposure to CYP and deltamethrin impaired

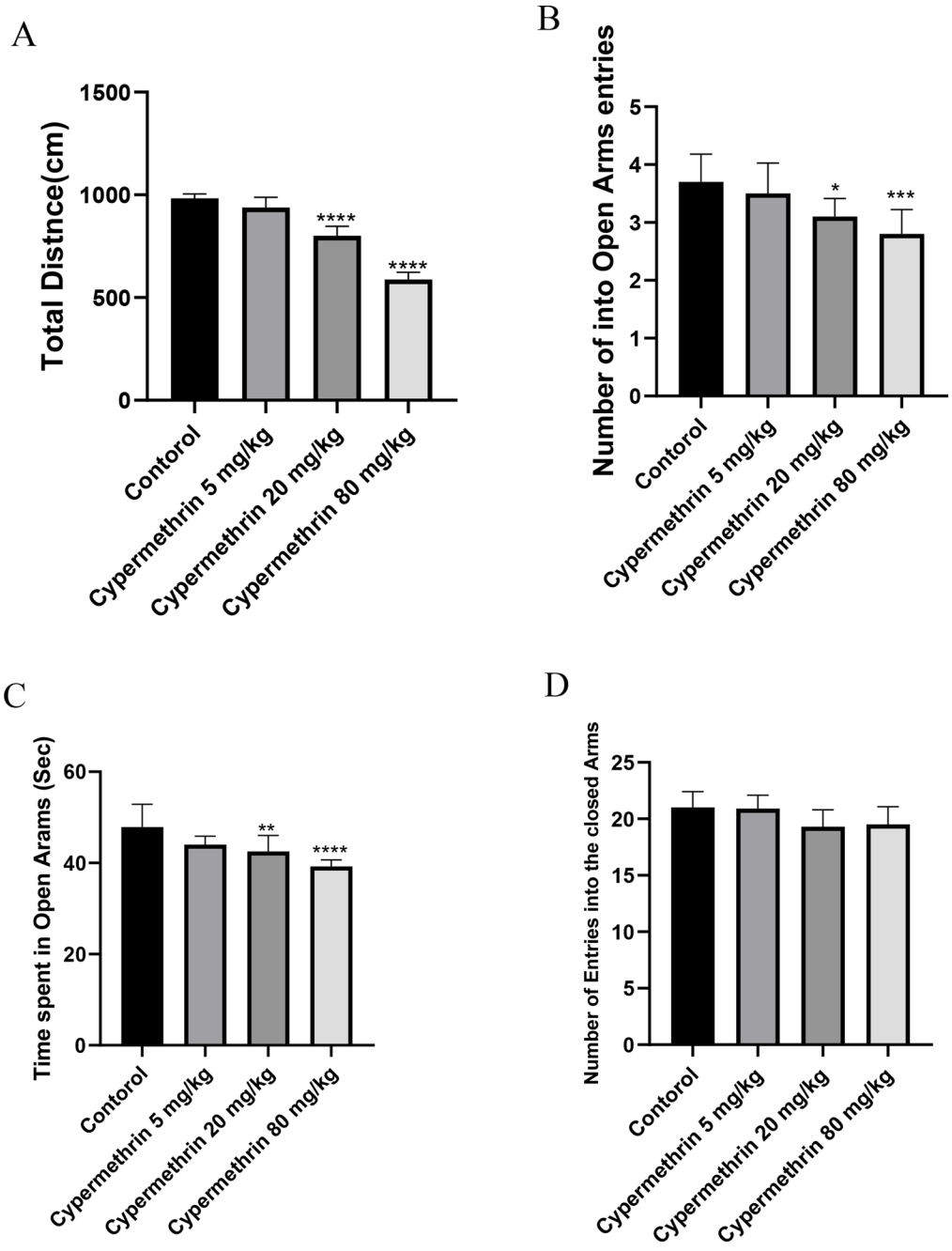

Fig. 5 The Effects of cypermethrin (5, 20, and 80 mg/kg) on elevated plus maze; the number of entrances into the closed arms (**A**), total distance moved (**B**), the number of entrances into open arms (**C**), and the number of entrances into the closed arms (**D**). Data are shown as mean ± SD for at least two independent experiments; $n = 10$ rats for each group. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and ****$P \leq 0.0001$ compared to the control groups. Significance refers to one-way ANOVA

corticogenesis [54]. Furthermore, CYP exposure during pregnancy has been shown to cause neurodevelopmental issues in children. CYP increases placental malondialdehyde (MDA) levels and the expression of genes associated with oxidative stress, which is significantly modified by environmental stress. Thus, the interaction between maternal CYP exposure and stress may have a significant impact on children's neurodevelopment [55].

Our study revealed that CYP exposure significantly impaired long-term spatial memory, as demonstrated by the MWM results, but did not significantly affect short-term memory in the NOR test. Several factors may explain this discrepancy. First, the differential sensitivity of the MWM and NOR tests to CYP-induced cognitive impairments could be a key factor. The MWM test primarily evaluates hippocampal-dependent spatial learning

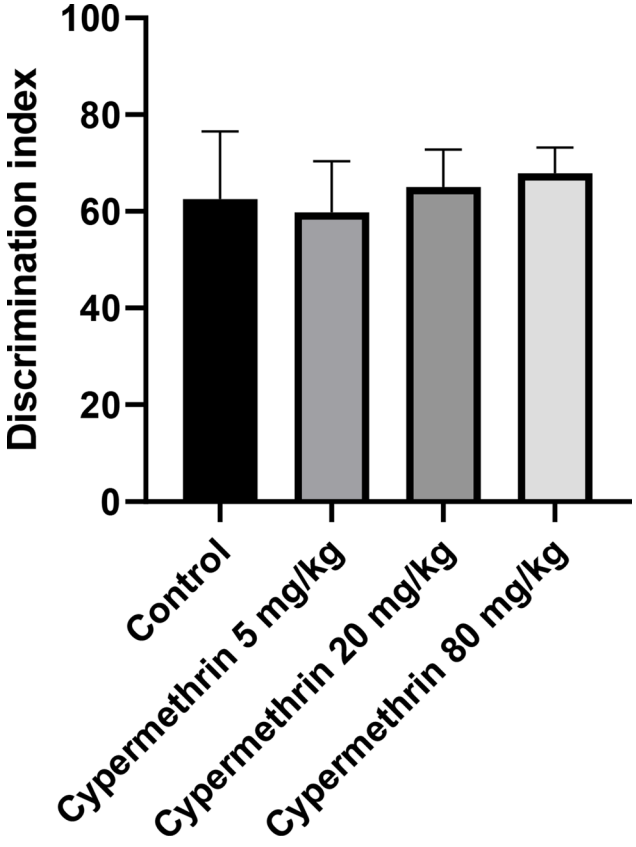

Fig. 6 The discrimination indices of the novel object recognition test between different groups. Data are shown as mean ± SD for at least two independent experiments; $n = 10$ rats for each group

and memory, which are more vulnerable to oxidative stress, apoptosis, and neuroinflammation—processes previously shown to be exacerbated by CYP exposure [53, 54]. In contrast, the NOR test assesses short-term recognition memory, which involves both the hippocampus and extrahippocampal regions, such as the perirhinal cortex. The damage caused by CYP exposure might be insufficient to significantly disrupt the neural circuits responsible for short-term memory within the study's timeframe or dose range. Second, methodological differences between the tests could contribute to the observed variability. The MWM test, with its complex spatial learning component and reliance on repeated trials, may be more sensitive to subtle cognitive deficits. On the other hand, the NOR test involves a shorter duration and simpler task structure, which could reduce its sensitivity to detecting mild cognitive impairments under certain conditions. Finally, task-specific effects of CYP might account for the differences. Long-term memory tasks, such as MWM, often require sustained attention, memory consolidation, and retrieval processes, which may be more susceptible to disruptions from neurotoxic agents like CYP. In contrast, short-term memory tasks, like the NOR test, might not be as heavily affected by these disruptions within the same experimental parameters. Further studies integrating molecular and histopathological analyses could provide deeper insights into the differential impact of CYP on various cognitive domains.

We also investigated the impact of CYP on locomotor activity. Our findings suggest that CYP administration resulted in reduced locomotor and rearing frequencies and increased immobility duration. Specifically, our results demonstrated that CYP induced a dose-dependent decrease in general motor activity, with the 5 and 20 mg/kg doses not significantly affecting motor function. Previous studies have shown that all PYRs affect motor function, regardless of species or the route of administration, making it one of the most important neurobehavioral endpoints for assessing PYR poisoning [56]. Most PYRs cause a dose-dependent reduction in general motor activity in mammals [56]. Therefore, it can be concluded that the effects of CYP on motor function are dose-dependent, and the results observed in other studies are closely aligned with our own [56, 57]. Choreiform movements of the limbs and tail, which are characteristic of type II acute PYR poisoning in rodents [12, 58, 59] lead to impaired movement coordination. In our study, we administered CYP at a low dose (5 mg/kg), and impaired movement coordination was observed. Similar results were reported by Nieradko-Iwanicka et al. [4]. The reductions in locomotor behavior may confound the effects observed in the other learning and memory endpoints. All the endpoints intended to evaluate learning and memory in our study are closely tied to motor function, linking our measurements of learning and memory to the animals' ability to move and explore their environment.

Evidence suggests that CYP affects the central nervous system (CNS) and causes neurological damage. As shown in previous studies, even a single exposure to CYP can lead to apoptosis in the CNS of affected animals [60]. The initial impact of CYP on the CNS is cortical neuron apoptosis through the Nrf2/ARE signaling pathway [60–63]. Histological and immunofluorescence methods have indicated the presence of apoptotic cells in the zebrafish retina following nine days of CYP exposure. Histone γ-H2AX, a marker of DNA damage, was observed in both the outer and inner nuclear layers, and caspase-3, an apoptotic marker, was found in the outer nuclear layer. These findings suggest that CYP can cause oxidative stress, DNA damage, and apoptosis in retinal cells [64]. CYP exposure was also found to decrease superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione (GSH), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), and total protein (TP) levels, while increasing lipid peroxidation (LPO) in rat serum [65]. In another study, significant reductions in SOD, CAT, and GPx enzyme activities were observed following CYP exposure, along with a significant increase

in MDA levels, a marker of lipid peroxidation [66]. CYP exposure also increased apoptosis signals, particularly in granulosa cells, and elevated the expression levels of Caspase 3, Bax, and Bcl-2. In granulosa KGN cells, CYP induced apoptosis, abnormal ROS production, and depolarization of the mitochondrial membrane potential compared to the control group [67]. Moreover, CYP induced oxidative stress and DNA damage in gill cells, leading to alterations in the activities of enzymes involved in maintaining ROS balance, as well as changes in their corresponding gene expression levels [68].

CYP can activate microglia and induce the release of IL-1β and TNF-α, while also upregulating the expression of PKC-δ, iNOS, phosphorylated p38, MMP-3, p42/44 MAPKs, and MMP-9 proteins, contributing to neurodegeneration [69]. IL-6 causes ligand-independent activation of the androgen receptor (AR), and CYP inhibits IL-6-mediated ligand-independent AR signaling. This provides new insight into CYP's antagonism of IL-6-associated ligand-independent AR activation [70]. Administration of β-CYP decreases glutamate concentration in the mouse cerebral cortex, leading to reduced glutamine synthetase activity and increased synaptosomal glutamate uptake. This evidence partially explains the neurotoxicity of synthetic β-CYP insecticides [71]. Oxidative stress plays a key role in Parkinson's disease, and chronic exposure to moderate doses of CYP can induce Parkinsonism. Another study showed that CYP altered oxidative stress indicators and impaired the antioxidant defense system in peripheral blood, providing insight into the nigrostriatal (NST) toxicity associated with Parkinsonism [72]. CYP exposure resulted in increased nigral dopaminergic neurodegeneration and microglial activation, while decreasing complex I activity and mitochondrial membrane potential. Western blot analysis revealed that CYP enhanced the expression of c-Jun N-terminal kinase (JNK), caspase-3, tumor suppressor protein (p53), p38 MAPK, TNF-α, and heme oxygenase-1 (HO-1), while reducing Bcl-2 expression. Additionally, CYP can induce mitochondrial dysfunction, alter the mitochondrial proteome, and lead to oxidative stress and apoptosis, contributing to the regulation of NST dopaminergic neurodegeneration [73]. The effects of chronic, acute, adulthood, and developmental exposures to CYP have been studied in experimental animals, providing further understanding of the alterations CYP induces in the CNS, particularly in relation to NST dopaminergic neurodegeneration. Comparisons between CYP-induced NST neurodegeneration and models of sporadic and chemically induced diseases, including their advantages and limitations, have also been explored [74]. CYP may also impair the migration of GABAergic progenitors, causing various transcriptional changes, both independently and in combination with stress [55].

Limitations

One limitation of our study was the over-testing of effects, as the rats had no rest days between tasks. Another limitation was the lack of molecular studies on the rats' brains, which would have allowed for a more precise determination of the molecular basis behind the observed behaviors, specifically in hippocampal or non-hippocampal regions. Therefore, additional experiments to investigate some of the potential underlying mechanisms would be highly beneficial. Although we restricted the study to one sex to avoid the confounding effects of hormonal fluctuations and included only adult specimens, a further limitation was the exclusive inclusion of male rats. Future studies could examine whether previous literature suggests any sex differences in the impact of CYP exposure.

Conclusion

In this study, we examined the effects of the CYP toxin on learning and memory in an animal model. The observed impairments in memory and learning may be attributed to the toxin's stimulatory effects on oxidative stress, apoptosis, and inflammation. Our results contribute valuable insights into the harmful impacts of CYP and provide clear evidence for limiting its widespread use. Further studies are needed to confirm the long-term detrimental effects of CYP on human health.

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Author contributions

A.K. statistical analysis of data, study design and supervision, writing and critical revision of the manuscript; M.N., M.S., A.S., and S.A.A. performing experiments, drafting the manuscript and data acquisition; A.Z. and S.R. administrative, material and technical support and critical revision of the manuscript for important intellectual content; N.F. manuscript drafting and technical support. All authors read and approved the final manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

The Ethics Committee of Hamadan University of Medical Sciences approved this research (IR.UMSHA.REC.1396.102).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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