Original Article

Effects of Occupational Formaldehyde Exposure on Passive Avoidance Conditioning and Anxiety Levels in Wistar Rats

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How to Cite This Article Resae, A., Yousefi, M. H., Naeimi, S., and Mahdavi, A. (2023). Effects of Occupational Formaldehyde Exposure on Passive Avoidance Conditioning and Anxiety Levels in Wistar Rats. *Iranian Journal of Veterinary Medicine, 17*(1): 65-74. http://dx.doi.org/10.32598/ijvm.17.1.1005241

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ABSTRACT

Background: Formaldehyde is a volatile organic compound widely used in industry and medical fields such as Anatomy and Pathology. Exposure to this chemical negatively affects the skin, mucous membrane, and respiratory system. It can pass through the blood-brain barrier, potentially causing neurotoxicity. According to studies, formaldehyde might be involved in memory impairment and the cognitive decline process in Alzheimer disease (AD).

Objectives: This study aimed to simulate chronic occupational formaldehyde exposure in rats and study its impacts on passive avoidance conditioning and anxiety.

Methods: Twenty-four adult male Wistar rats were divided into four groups of 6 rats each. After an adaptation period, the rats were exposed to 1, 2, and 3 ppm formaldehyde vapor in an exposure chamber, 6 hours per day for 7 days. The control group was exposed to saline. After the exposure period, a shuttle box for passive avoidance conditioning and an elevated plus-maze test for assessing anxiety levels were performed. The data were analyzed by 1-way ANOVA and Duncan's multiple range test for group comparison in SPSS and SAS software.

Results: In the shuttle box test, formaldehyde dose-dependently decreased escape-through latency and increased the percentage of dark compartment entries ($P<0.0001$). In the elevated plus maze test, the percentage of time spent in open arms decreased by increasing the dosage ($P<0.0001$).

Conclusion: Based on these findings, formaldehyde exposure can negatively alter brain function and cause memory impairment and anxiety.

Keywords: Anxiety, Formaldehyde, Memory, Rat, Shuttle box

Article info:
Received: 07 Jun 2022
Accepted: 22 Aug 2022
Publish: 01 Jan 2023

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1. Introduction

Formaldehyde is a volatile organic compound (VOC) and a well-known indoor and outdoor pollutant found in paint, clothes, cigarette smoke, and exhaust gas due to incomplete combustion. It is widely used in various industries and poses a significant health problem in industrial nations (Qu et al., 2017). It is also used as a preservative for cadavers, tissues, and specimens in medical fields such as Anatomy and Pathology. Faculty members and students, researchers, anatomists, and technicians working in dissection halls are continually exposed to the toxic vapors of formaldehyde (Nogueira et al., 1997).

Exogenous formaldehyde is mainly absorbed from the respiratory system. Formaldehyde is highly electrophilic and can interact easily with DNA and proteins forming toxic methylene bridges and causing genotoxicity (Reingruber and Pontel, 2018). It can also pass through the blood-brain barrier and enter the central nervous system, causing neuronal irritation (Li et al., 2016a). Therefore, it is potentially neurotoxic.

In humans, long-term and chronic formaldehyde exposures may lead to multiple chemical sensitivity or sick-building syndrome. Some common symptoms of multiple chemical sensitivity are anxiety, depression, headache, fatigue, weakness, memory and concentration difficulties, irritability, muscle and joint pain, gastrointestinal problems, and upper airway irritation. Most of these symptoms probably occur due to sensitization of the limbic system. Hypothalamic-pituitary-adrenal axis or the corticosterone level alteration induced by formaldehyde can potentially cause nervous system damage. Increased fear, anxiety, and altered cortisol levels in humans have been associated with clinical syndromes, such as post-traumatic stress disorder and depression (Sorg et al., 2004).

The prefrontal cortex and the limbic system, particularly the amygdala and hippocampus, play important roles in anxiety and emotional reactions. Amygdala is involved in stress, anxiety, fear, and conditioning. Hippocampus also plays an important role in the process of memory and learning. In addition, other systems, such as serotonergic, noradrenergic, and dopaminergic inputs from the raphe nuclei, locus coeruleus, and ventral tegmental area, can also influence behavior and anxiety (Canteras et al., 2009).

Rodents and humans have more than 90% of common genes involved in anxiety and the brain system (Kumar et al., 2013). This system includes the defensive system, showing immediate responses to threatening stimuli, behavioral control, and avoidance (Canteras et al., 2010).

Emotions, memory, and behavior mostly emerge from the limbic system, a group of interconnected structures in the brain. Damages or changes in the limbic system can result in dysfunction in various behavioral and cognitive areas (Cardinali, 2018). Electrophysiological studies have shown that the hippocampus is involved with the learning process, and lesions in this area have previously caused learning and memory deficits in animal test subjects (Donkelaar et al., 2020). Similarly, patients with hippocampal damage showed impaired memory and performed poorly in maze learning, working memory, and object-locating tasks (Clark and Maguire, 2016).

The permissible occupational exposure limit for formaldehyde is 0.75 ppm, measured as an 8-h time-weighted average (OSHA, 2011). However, previous studies have shown the formaldehyde concentration varying between 0.75 and 4 ppm inside anatomy laboratories, embalming rooms, and personal sampling rooms (Albertini and Kaden, 2017; de Lucena et al., 2017). In industry, the concentration of formaldehyde exposure is reported between 0.04 and 3.48 ppm, with wood production workers, mortuary employees, and firefighters exposed to the highest concentrations (Dan et al., 2020). Long-term exposure to these formaldehyde levels may seriously damage people. For example, in one study, formaldehyde exposure had adverse effects on hematopoiesis in industrial workers and made them more susceptible to leukemia (Kang et al., 2021).

In the present study, we investigated the effects of repeated formaldehyde exposures on passive avoidance conditioning, a form of memory, risk-taking behavior, and anxiety levels in rats. The experimental doses were selected by the human occupational formaldehyde exposure trends (Nogueira et al., 1997), and we tried to simulate human occupational formaldehyde exposure in this study.

In the current study, the effects of chronic formaldehyde exposure on anxiety levels and passive avoidance conditioning, a form of memory in Wistar rats, were investigated. This study aimed to test whether the rats would react to experimental exposures similar to the occupational concentrations of formaldehyde. We hypothesized that chronic formaldehyde exposure would induce memory impairment and alter behavior. To test

our hypothesis, a shuttle box and an elevated plus maze (EPM) were included. We chose these tests because they are standard tests for memory and behavior assessment.

2. Materials and Methods

Study animals

Twenty-four adult male Wistar rats (about 60 days old) with a mean weight of 0.18-0.2 kg were purchased from Shahmirzad Laboratory Animals' Research Center and brought to the Behavioral Research Laboratory of Semnan Veterinary University. The animals were housed in groups of 6 in large transparent Makrolon cages for one week before the experiment for adaptation. The rats were kept at a constant temperature of about 22°C±2°C and a 12:12 h light:dark cycle and had full access to standard laboratory food and water supplies.

Exposure procedure

The rats were divided into four groups of 6 (control group, 1 ppm, 2 ppm, and 3 ppm formaldehyde exposure groups). Formaldehyde (37%) was purchased from Arvin Shimi Delta Chemical Lab (IR). Exposure took place in an exposure chamber with 0.51×0.51×0.98 m dimensions. The box was covered with thick plastic film to maintain a stable internal condition. The chamber environment was monitored continuously with a portable oxygen analyzer (model Kane 510; Keison Products, Chelmsford, England). At first, one rat was placed in the chamber for 6 h as a pilot experiment to ensure the safety and health of the animals in such conditions. The formaldehyde vapor dose needed for each group was calculated according to the United States Environmental Protection Agency (EPA) formula as mg/m^3=(ppm)×(molecular weight of the compound)/ (24.45) (Salonen et al., 2020), and the exposure chamber dimensions. The exposure time was from 8:30 AM to 2:30 PM every day for 7 consecutive days. The animals did not have any food or water access throughout the exposure times and were monitored carefully. Behavioral tests were carried out one day after the end of the exposure period. The experimental doses were selected by the human occupational formaldehyde exposure trends (Nogueira et al., 1997), and this study aimed to simulate human occupational formaldehyde exposure in this study.

Behavioral tests

After the adaptation, exposure, and training periods, the main tests (shuttle box test and EPM) were performed.

Shuttle box

This test was selected for assessing the passive-avoidance conditioning behavior in the rats. The apparatus consisted of a light and a dark compartment. A guillotine door separated the compartments. The floor of the dark compartment was coated with a stainless steel grid floor, delivering an electric shock with a stimulator (1 mA, 50 Hz). In the training or adaptation session, the rats were placed in the light compartment, and the guillotine door was raised 10 s afterward. Upon entry to the dark compartment, the door was closed, and the animals were removed from that section. If the entry to the dark section did not happen during the first 100 s, the animal would be eliminated. The acquisition stage was performed 30 minutes after training. Rats were placed into the light compartment, and the guillotine door was raised 10 s later. The guillotine door was closed upon full entry to the dark compartment, and the animals received an electric shock. The dark compartment entry time was noted. The retention test was performed 24 hours after acquisition. The guillotine door was raised 10 s after placing the rats in the light compartment. The entrance time to the dark section was noted as escape-through latency. When the animals did not enter the dark compartment for a maximum of 300 s, successful acquisition of the passive avoidance response was recorded. Shorter latencies indicated poor retention.

Elevated plus maze

EPM is a test for measuring anxiety levels in laboratory animals. The platform consisted of two open and two closed arms connecting through a central platform. The apparatus was elevated 0.5 m above the ground. Rats were placed in the central area, and a camera recorded their movements for 5 min. The data were then analyzed. In the present study, the percentage of the total time spent in open arms was measured. The area was cleaned after each test. The percentage of the time spent in open arms was calculated by the formula (Time percentage spent in open arms=time spent in open arms/ time spent in open arms+time spent in closed arms×100) for each group (Kapogiannatou et al., 2016).

Data analysis

The parametric data were analyzed using SAS software, version 9 (2/2009 in the GLM procedure). The analyses included analysis of variances and differences between treatments using Duncan's multiple range tests. The nonparametric data were analyzed by IBM Corp. SPSS (IBM Ver. 21/ 2012) by calculating the mean and using the Friedman test to compare the ranks.

3. Results

Shuttle box

The escape-through latency graph (Figure 1a; Table 1) demonstrates a statistically significant decrease in the entry time to the dark section of the shuttle box in formaldehyde-exposed groups in comparison with the control group in the retention test (P<0.0001). We also observed a significant decrease in the acquisition-retention avoidance percentage (percentage of the rats avoiding the dark section 24 hours after receiving an electric shock) in formaldehyde-exposed groups (Figure 1a; Table 1) (P<0.0001). However, no significant difference was found between the 1-ppm and 2-ppm groups (P<0.0001).

Elevated plus maze

Based on the EPM test results (Figure 2; Table 1), formaldehyde exposure decreased the percentage of time spent in open arms in the exposure groups. There was a significant decrease in this parameter in the 2-ppm and 3-ppm exposure groups compared with the control group (P<0.0001). The decrease was not statistically significant in the 1-ppm group compared with the control group (P>0.0001).

Clinical observations

No significant changes in food or water intake were observed. The rats exposed to formaldehyde displayed symptoms of respiratory irritation, such as coughing and sneezing during the exposure sessions. Yellow discoloration of the fur was observed in the formaldehyde-exposed groups (Figure 3; Table 2; Table 3). The yellowing of the fur was also documented by some previous investigations and is said to arise from the reaction between formaldehyde and kynurenine, which is a natural component of the fur in rats (Til et al., 1988). We graded the discoloration from 1 to 3 (with 1 being nonexistent and 3 as severe discoloration) in the test groups.

Table 1. Effects of formaldehyde exposure on various parameters in shuttle box and elevated plus maze (EPM)

Exposure Groups	Mean±SE		
	Shuttle Box (S)	Shuttle Box (%)	EPM (Open Arms %)
Control	300.00^{a}±0.00	100.00^{a}±0.00	39.00^{a}±2.42
1 ppm	6.50^{b}±0.35	33.00^{b}±1.64	37.83^{a}±3.07
2 ppm	6.17^{b}±0.32	33.00^{b}±1.45	27.33^{b}±3.09
3 ppm	4.67^{b}±0.40	17.00^{c}±1.19	22.50^{b}±2.20
SEM	22.58	5.77	1.86
P	<0.0001	<0.0001	<0.0001

a,^{b}: Different letters indicate statistically significant differences between the groups.

Table 2. Grading of the Fur discoloration caused by formaldehyde

Groups	Max	Min	Mean Rank	Mean±SE
Control	1	0	1.19a	0.25±0.53
1 ppm	2	1	2.44b	1.38±0.51
2 ppm	2	1	2.69b	1.63±0.52
3 ppm	3	2	3.69c	2.5±0.46

a,b,c: Different letters indicate statistically significant differences between the groups.

Table 3. Asymptotic significance in the chi-square test

Variables	Control	1 ppm	2 ppm	3 ppm
Control	--	0.08	0.014	0.005
1 ppm	0.08	--	0.317	0.014
2 ppm	0.014	0.317	--	0.025
3 ppm	0.005	0.014	0.025	--

4. Discussion

We evaluated the effects of formaldehyde exposure on anxiety, memory, and passive-avoidance conditioning, a form of associative memory.

Conditioning is a form of associative implicit memory in which the brain forms connections between initially unrelated items or events (Trask et al., 2021). The animals learn how to predict future events to express proper behavior through the consequences of their actions and make connections between the stimuli and the responses (Quillfeldt, 2016). This aspect of memory and learning is critically important in everyday life. Unfortunately, it is also one of the first aspects of memory performance impacted by aging and Alzheimer disease (AD) (Matzen et al., 2015).

In the shuttle box retention test, the formaldehyde-exposed rats entered the dark compartment more than the control group rats, and the latency time to enter the dark compartment decreased. These effects were dose-dependent (Figure 1; Table 1). These findings were consistent with Makowski's study in which the formaldehyde-derived tetrahydroisoquinoline temporarily induced memory impairment in the active-avoidance test (Makowski and Ordonez, 1981). Therefore, we can conclude that formaldehyde exposure impairs memory for passive and active avoidance conditioning tasks.

The increased errors of the formaldehyde-exposed rats in the shuttle box can be interpreted in two ways. Either the rats did not remember the shock due to memory impairment, or they failed to acquire avoidance due to hyposensitivity and impaired nociceptive behavior.

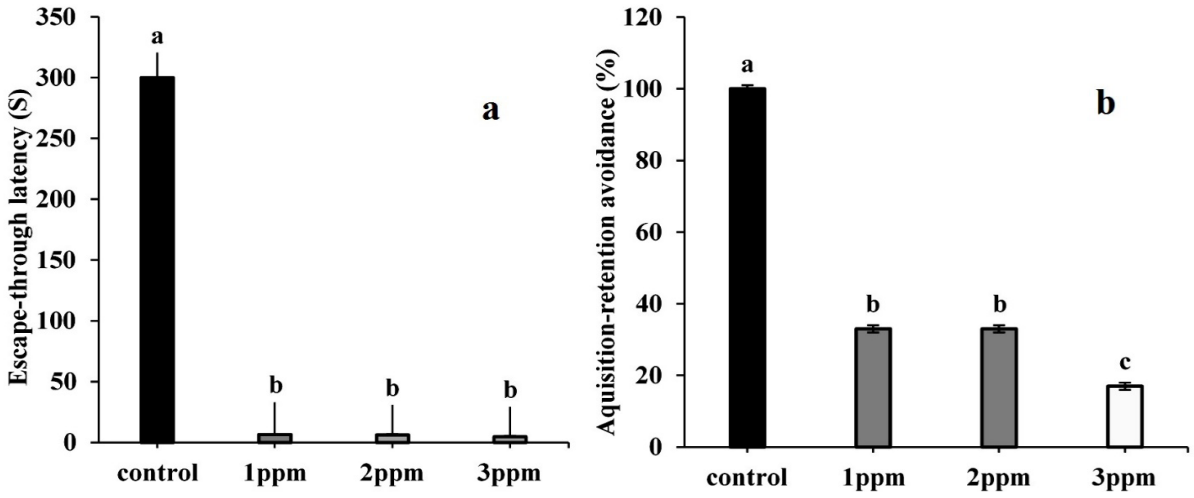

Figure 1. Shuttle box apparatus

a) Time from releasing the rats in the starting position to stepping into the dark compartment (Escape-through latency) in the shuttle-box apparatus. The Y-axis represents the escape through latency time (S), while the X-axis represents the formaldehyde doses. b) The percentage of the rats not entering the dark compartment 24 hours after the administration of electric shock delivered in the shuttle-box apparatus (retention test). The Y-axis represents the animals not entering the dark compartment (%), while the X-axis represents the formaldehyde doses. Different letters indicate statistically significant differences between the groups (P<0.0001).

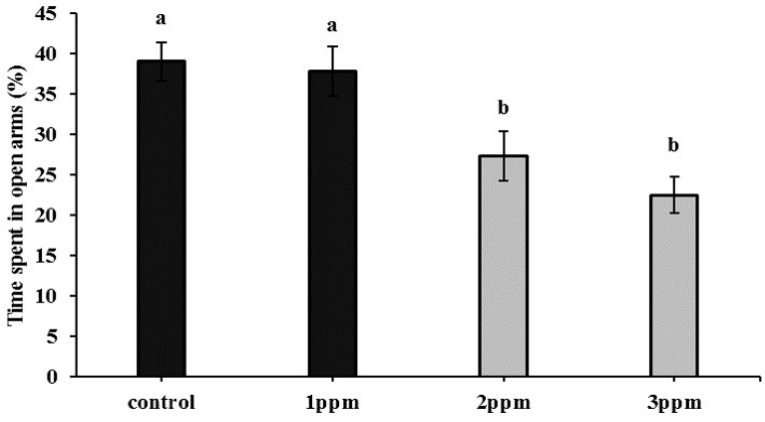

Figure 2. Percentage of the time spent in the open arms in elevated plus maze (EPM) within five minutes

The Y-axis represents the time percentage, while the X-axis represents the formaldehyde doses. Different letters indicate statistically significant differences between the groups (P<0.0001).

Both hippocampus and amygdala play important roles in fear conditioning. The hippocampus may relay environmental inputs related to the conditioning context to the amygdala (Korte and De Boer, 2003). Amygdala can create and store long-term memory by processing emotional information and stimuli. It is involved in the consolidation of strong emotions and fear memory. In many cases, the number of reactivated neurons in the amygdala and the strength of the retrieved fear memory are correlated (Josselyn, 2010). It is documented that inducing damage to the amygdala, particularly in the central nucleus region, leads to impaired acquisition in passive avoidance tests. The connections between the infralimbic cortex and the central nucleus of the amygdala may be involved in the ability of the rodents to associate aversive nociceptive stimuli, such as shock, with other sensory stimuli, such as visual clues in avoidance tasks. According to studies, infralimbic lesions can lead to deficits in passive avoidance conditioning tasks (Capuzzo and Floresco, 2020). In addition, ventral hippocampus lesions have also impaired fear conditioning (McDonald et al., 2018). In the shuttle box, entering the dark section 24 hours after electric shock administration could result in memory impairment. Therefore, our findings demonstrate that formaldehyde exposure can induce memory impairment in conditioning tasks.

Figure 3. Yellow discoloration of the fur caused by formaldehyde

The control group rat with normal white fur (a), and the 3-ppm exposure group rat with yellow fur (b).

Formaldehyde can pass through the blood-brain barrier. This compound is highly reactive and can cross-link with proteins, unsaturated fatty acids, DNA, and RNA. Therefore, it can alter brain function, leading to neurotoxicity (Reingruber and Pontel, 2018).

For example, the N-methyl-D-aspartate (NMDA) receptor is a ligand-gated ion channel that plays a significant part in neuronal plasticity in the nervous system (Deng et al., 2019). A greater hippocampal synaptic efficacy originating from an increased density of NMDA receptors can improve the performance in memory-related tasks, while the blockade of them by NMDA antagonists can induce learning and memory deficit (Lu et al., 2008). Formaldehyde exposure can block the NMDA receptors by down-regulating the expression of NMDA receptor subunits (Tong et al., 2013).

NMDA receptor antagonists such as dizocilpine (MK-801) have previously been shown to induce learning and memory impairment in Morris water maze (MWM) test in rats. The rats needed more time to complete maze tests and made more mistakes (Svalbe et al., 2019). According to studies, selective serotonin 5-HT7 and 5-HT1A receptor antagonists can reverse MK-801-induced learning and memory impairment (Horisawa et al., 2011). In one study, intraperitoneal administration of formaldehyde decreased the 5-hydroxytryptamine (5-HT) levels and memory function, negatively affecting the shuttle-box results (Li et al., 2016a).

NMDA receptors also take part in nociceptive behavior. Administration of NMDA agonists can result in nociceptive behavior that can be attenuated by NMDA receptor antagonists (Deng et al., 2019). Therefore, the hyposensitivity of the rats to the shock and formaldehyde-induced lack of nociceptive behavior in exposure groups might be an alternative reason behind the deficit observed in the shuttle box test.

By contrast, some studies imply that low-dose and short-term exposure to formaldehyde can excite the central nervous system and temporarily improve avoidance conditioning memory in the tests in another study (Sorg et al., 2004). However, in our study, chronic exposure to formaldehyde for one week significantly impaired memory and increased errors in the shuttle box.

Changes in corticosteroid receptors might be another reason behind the memory impairment caused by formaldehyde. Corticosteroids affect memory and emotional behavior mainly via the central amygdala, stria terminalis, and hippocampus (Korte and De Boer, 2003).

Studies have mentioned behavioral and cognitive changes induced by increased oxidative stress in the brain due to VOCs, such as toluene, benzene, and formaldehyde. For instance, exposure to toluene can increase lipid peroxidation, oxidized proteins such as protein carbonyls, and mitochondrial (UBIQ-RD) and cytosolic NAD(P)H:quinone oxidoreductase 1 (NQO1) markers for reactive oxygen species in various brain regions (Kodavanti et al., 2011). Formaldehyde and Toluene have also increased free radicals and malondialdehyde levels from lipid peroxidation and decreased superoxide dismutase and glutathione functions, two catalyzing agents in the antioxidant activities in the central nervous system (Lu et al., 2008).

In our experiment, formaldehyde exposure decreased the time spent in the open arms of the EPM (Figure 2; Table 1). This reaction was dose-dependent, which indicates that by increasing the exposure dose, the time spent in open arms decreased compared to the control group. Our findings were consistent with prior studies (Li et al., 2016b).

EPM is a widely used test for measuring anxiety levels, risk assessment, and exploratory and avoidance behavior of rodents in the laboratory. In the EPM test, anxiety is expressed by animals spending more time in closed arms. While the less anxious individuals are more willing to take risks, explore and spend more time in open arms (Quillfeldt, 2016). Anxiolytic and anxiogenic drugs can alter the EPM results. Anxiolytic drugs such as diazepam increase risk-taking behavior and open-arm entries, while anxiogenic drugs like pentylenetetrazol decrease open-arm entries in tests (Bertoglio and Carobrez, 2016). In our study, formaldehyde exposure decreased the time spent in open arms. We can conclude that formaldehyde exposure increases anxiety levels and inhibits curiosity and exploratory behavior.

Rats' function in EPM and their increased anxiety levels may be related to the concentration of the receptors in the limbic system, serotonin lateralization in the amygdala, and dopamine elevation and lateralization in the prefrontal cortex in the brain (Anderson and Teicher, 1999).

In one EPM experiment, dorsal and ventral hippocampal injection of meta-chlorophenylpiperazine, a 5-HT serotonergic receptor agonist, did not affect anxiety levels or locomotor behavior in mice, while its 1.0 nMol amygdalar injection induced anxiety. The behavioral alterations could also be inhibited by injecting 5-HT receptor antagonists. These results imply that the amygdala 5-HT

receptors play an important part in anxiety and stress-induced behavior (Cornelio and Nunes-de-Souza, 2007).

Neurons within the amygdala express a high density of corticosteroid receptors. The central nucleus of the amygdala can also facilitate the activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress (Wiktorowska et al., 2021). Formaldehyde could increase glucocorticoid levels by triggering and exciting the HPA axis. Prolonged repeated exposures to glucocorticoids or VOCs such as toluene and formaldehyde can act as stressors and downregulate the number of central glucocorticoid receptors in the brain, predominantly in the hippocampus and amygdala. These changes lead to negative feedback, which then elevates the corticosterone levels. This process may lead to formaldehyde-induced chronic anxiety and depression (Korte and De Boer, 2003; Li et al., 2016b).

5. Conclusions

Overall, chronic exposure to formaldehyde impairs passive avoidance conditioning in the shuttle box and decreases the time spent open arms in the EPM test in rats. Increased errors in the shuttle box could be a result of impaired memory, impaired nociception, or increased risk-taking behavior. However, in EPM, the rats exposed to formaldehyde spent less time in open arms and displayed a preference for closed arms without the locomotor activity being impaired. This event demonstrates risk-averse behavior and increased anxiety. Therefore, it is highly unlikely that the latter theory is correct. Our findings confirm the neurotoxicity of formaldehyde and demonstrate that repetitive formaldehyde exposures can increase anxiety and stress-related behavior in a dose-dependent manner.

Ethical Considerations

Compliance with ethical guidelines

The maintenance and treatment of the animals were in accordance with the care and use of the laboratory animals' committee guidelines of Semnan University, and we received the code 67-23-7-97 from the National Animal Research Ethics Committee.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to express their sincere gratitude towards the Deanship of the Faculty of Veterinary Medicine, Semnan University, for providing facilities to perform this research.

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مقاله پژوهشی

تأثیر مواجهه با فرمالدهید شغلی بر شرایط احترازی غیرفعال و سطوح اضطراب در موش‌های صحرایی ویستار

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How to Cite This Article Resae, A., Yousefi, M. H., Naeimi, S., and Mahdavi, A. (2023). Effects of Occupational Formaldehyde Exposure on Passive Avoidance Conditioning and Anxiety Levels in Wistar Rats. *Iranian Journal of Veterinary Medicine, 17*(1): 65-74. http://dx.doi.org/10.32598/ijvm.17.1.1005241

http://dx.doi.org/10.32598/ijvm.17.1.1005241

چکیده

زمینه مطالعه: فرمالدهید یک ماده‌ی آلی فرار بوده که در صنعت و پزشکی کاربرد فراوان دارد. این ماده بر پوست، مخاطات و تنفس اثر گذاشته و طبق برخی مطالعات، فرمالدهید توان ورود به مغز و ایجاد عوارض عصبی داشته و در پروسه‌ی آلزایمر نیز دخالت دارد.

هدف: هدف از این مطالعه شبیه‌سازی مواجهه‌ی شغلی با فرمالدهید و بررسی اثر آن بر شرطی‌شدن احترازی غیرفعال و میزان اضطراب در موش صحرایی بود.

روش کار: در این مطالعه ۲۴ قطعه رت بالغ نژاد ویستار به چهار گروه تقسیم شدند. گروه‌های تحت درمان پس از دوره‌ی سازش پذیری، به‌مدت هفت روز متوالی و روزانه شش ساعت مورد مواجهه با فرمالدهید با دوزهای ۱، ۲ و ۳ ppm قرار گرفتند. در گروه کنترل به‌جای فرمالدهید از نرمال سالین استفاده شد. پس از اتمام دوره‌ی مواجهه، تست‌های رفتاری شاتل‌باکس و ماز بعلاوه‌مرتفع انجام شد. برای آنالیز داده‌های آماری از نرم افزار SPSS و SAS، و برای مقایسه‌ی گروه‌ها از آزمون آماری ANOVA و آزمون تکمیلی دانکن استفاده شد.

نتایج: مواجهه با فرمالدهید موجب افزایش درصدی زمان سپری شده در بازوی بسته در آزمون ماز بعلاوه مرتفع گردید (P<۰,۰۰۱). همچنین در آزمون شاتل‌باکس، مواجهه با فرمالدهید موجب افزایش درصد و کاهش تاخیر زمانی ورود حیوانات به اتاقک تاریک شد (P<۰,۰۰۰۱).

نتیجه‌گیری نهایی: با توجه به یافته‌های مطالعه‌ی حاضر، مواجهه با فرمالدهید بصورت استنشاقی می‌تواند بر دستگاه عصبی مرکزی و حافظه اثر مخرب داشته و اضطراب را نیز افزایش دهد.

کلیدواژه‌ها: اضطراب، فرمالدهید، حافظه، موش صحرایی، شاتل باکس

تاریخ دریافت: ۱۷ خرداد ۱۴۰۱
تاریخ پذیرش: ۳۱ مرداد ۱۴۰۱
تاریخ انتشار: ۱۱ دی ۱۴۰۱

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